Evaluation of Imprint Cytodiagnosis in Cases of Lymphadenopathy & Comparative Study with Frozen Section and Paraffin Section

THESIS

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C E R T I F I C A T E

This is to certify that the work entitled "EVALUATION OF IMPRINT CYTODIAGNOSIS IN CASES OF LYMPHADENOPATHY AND COMPARATIVE STUDY WITH FROZEN SECTION AND PARAFFIN SECTION", which is being submitted as a thesis for M.D. (Pathology) by DR. DILIP KUMAR, has been carried out in the Department of Pathology, M.L.B. Medical College, Jhansi.

He has fulfilled the necessary stay in the department as required by the regulation of the Bundelkhand University.

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Dated : 22.1.90

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C O N T E N T S

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I N T R O D U C T I O N

INTRODUCTION

Lymphnodes are catch basin or filters in the lymphatic drainge system and hence frequently enlarges, so their enlargement is most common presentation of various non malignant and malignant diseases and this is the reason why lymphnodes are biopsied in routine. Although not so common as the primary site of disease, they are involved virtually in all systemic infections and large number of malignant lesions. This makes the diagnosis of lymphadenopathy as one of the most common problems for a surgical pathologist.

Lymphnode is highly cellular organ, so that any delay in fixation results in poor preservation and leading to thick section, so that histopathological features are altered. Another cause of altered histology is that lymphnode react to all external stimuli leading to extreme degree of hyperplastic changes, so the accurate diagnosis is difficult to make (Ackerman, 1954).

There are various methods by which lymphnodes are examined; Fine needle aspiration cytology, Imprint cytology, and forzen section for rapid diagnosis and paraffin section for final diagnosis.

Greig and Gray (1904) first punctured the lymphnode in search of etiological agent in case of trypnosomiasis.

Guthrie (1921) was the first person to use aspiration technique on enlarged lymphnode and its cytological finding were described by Fronker in 1927.

Martin and Ellis (1930) studied 1844 cases of cervical tumour. Various other workers like Stewart (1933) and Pavlousky's (1934) also made further research on this topic.

Berman (1953) studied the imprint cytology of the lymphnode and correlated it with histopathological findings and Dreyfus (1940) proved the advantage of Giemsa and Papanicolaou's staining for the lymphnode imprint.

By now though a large number of workers Dudgeon and Patrick (1927), Dudgeon and Barrett (1934), Lucas P.F. (1955), Ultamann et al (1958), Aust et al (1971), Godwin (1976), Bloustein and Silverbert (1977),Agarwal et al (1977), Solanki et al (1977), Suen et al (1976 & 1978), and Nagpal et al (1982) have critically evaluated and re-evaluated its diagnostic importance by imprint cytology method, but have not yet revealed universal acceptance. Various workers have stressed the utility of this technique on neoplastic and non neoplastic lesion

and most of these studies were however, confirmed to the lesions on lymphnodes. Therefore it was considered worth while to assess the value of imprint cytology in the diagnosis of lymphnode diseases.

This is a single retrail almost accurate procedure an adjunct to biopsy diagnosis. Some times also provides information of the histogenesis and pattern of tumour but is not helpful in assessing the depth of tumour infiltration.

Frozen section diagnosis has been useful surgical adjunct, since it was first popularised by Wilson in 1905 in the treatment of prostatic cancer, frozen section diagnosis during pelvic lymphnode staging biopsies are used.

This above technique is recent diagnostic procedure in pathology provides an quick tissue diagnosis, and quite simple technique and no fixative is needed so that there is not alteration in cellular morphology pattern of the lymphnode giving better and earlier results (Shivas and Fraser, 1971).

Because the diagnosis made by the pathologist with frozen section may have serious consequences for the treatment of the patient a high degree of accuracy is mandatory and quality control is important. The

surgeon constitutes a critical component of the diagnostic efforts by his selection of the tissue from which frozen section is to be done. No pathologist can over come the handicap of being handed the wrong tissue consequently, both the surgeon and pathologist must be advised of the each other problem and limitations of frozen section are concluded to give maximum services (Ewing, 1925; Simpson, 1937; Sparkman, 1952; Ackerman et al, 1959; Tribe, 1973; and Kaufman Zui et al, 1986). The paraffin embedded section technique is an old history of histopathology itself but inspite of certain pit fall its still resumes universal acceptance because the technique is comparatively cheap, simple and to a good amount of reliability (Culling, 1963; Bancroft and Steven, 1977).

MATERIAL AND METHODS

MATERIAL AND METHOD

The study for evaluation of imprint cytodiagnosis in case of lymphadenopathy and comparative study with frozen section and paraffin section was conducted on the patients having lymphadenopathy, attending the out patient department as well as admitted cases in the wards of M.L.B. Medical College Hospital, Jhansi, over period of one year extending from Sept 1988 to Oct 1989.

Before obtaining the lymph node, the patient of different age groups with obvious lymphadenopathies (localised and generalised) were throughly examined. Details of history and examination were recorded in a pre set working proforma.

Collection of lymphnode :

Palpable and enlarged lymphnode was removed by surgical procedure in operation theatre. The lymph node was brought to the laboratory without any delay. Gross features were recorded in terms of colour, size, shape, surface, consistancy, appearance of cut surface, presence of caseation, caleification, haemorrhage and any cystic degeneration. The lymphnode was divided into two halves; imprint smears were obtained from the cut surfaces and then on half was preserved in buffered formal saline and another was kept for frozen section.

Preparation of Imprint :

Lymphnode was bisected by sharp knife, cut surface of lymphnode was pressed gently against the surface of pre albuminised slides but without rubbing and series of imprint were taken. These slides were immediately fixed in ether and absolute alcohol (1:1). This ether alcohol mixture causes immediate precipitation of proteins without altering the morphological character of the collected material. This precipitated protein get adhere to the slide, thereby fixing the smear. Though only a few minute are necessary for fixation, the slides were kept in the fixative for a minimum period of half an hour, routinely to enhance completes fixation.

Staining of smear :

Two stains were used for the imprint cytology smears.

(i) Papanicolaou's stain.

(ii) Harris's haematoxylin and eosin stain.

Papanicolaou's staining (1942) :

This stain is developed by George Papanicolaou widely used in all sorts of cytological examination. It requires Harris's alum haematoxylin to stain the nucleus and OG-6 and polychrome stain EA-50 for staining the cytoplasm. Other reagents required 1% acid alcohol of different strengths and xylol. Steps of the technique were as follows :-

1. The fixed smears were at first carried through a process of hydration by dipping them for 30 seconds in each, descending grade of alcohol (80%, 70%,50%) to water.

2. The hydrated smears were then dipped in Harris's alum haematoxylin for 5 minutes.

3. The slides were then rinsed in tap water to wash the excess stain.

4. These slides were then carried through one or two dips in one percent acid alcohol for differentiation and decolourisation of cytoplasm and rinsed in tap water again.

5. The differentiated smears were then put into running tap water for 15 minutes for blueing.

6. The smears were then completely dehydrated again by carrying them through 50%, 60%. 70%, 80%, 90% and two changes of absolute alcohol for 30 second in each.

7. The completely dehydrated smears were then put into the jar containing the stain OG-6 and kept these for 2 minutes and then rinsed again in two changes of absolute alcohol.

8. The rinsed smears were then stained with the stain EA-50 by keeping them dipped into jar of stain for a period of 3 to 5 minutes.

i) The smears thus stained were rinsed in two changes of absolute alcohol once more.

ii) The rinsed stained smear were finally drained and cleaned in two changes of xylol.

iii) The smears were mounted in D.P.X.

Results of Papanicolaou's stain

Nuclei	-	Bluish violet
Acidophilic cells	-	Red to orange
Basophilic cells	-	Green to bluish green
Fragment of tissue	-	Orange to orange green
R.B.C.	-	Orange

Haematoxylin and Eosin staining :

The fixed smears were hydrated as already described and stained with haematoxyline by keeping them dipped in the stain for 5 minute. Smears were then washed with water to remove the excess stain and differentiated by dipping them once in 1% acid alcohol. When proper differentiation is acheived, the smears were dehydrated by passing them through ascending grades of alcohol as already described and then counter stained with 1% eosin for 30 seconds. The smears were then dipped in absolute alcohol to get rid of excess of eosin. The smears were then drained and cleaned in xylol and mounted in D.P.X.

Results of Haematoxylin and Eosin stain

Nuclei	--	Bluish violet colour
Cytoplasm	--	Orange to pink

Examination of the smear :

The mounted smears were now examined under low power and high power by light microscope. Beside looking for malignant cells, presence of other associated material in the smear like R.B.C., infiltrating cells, histiocytes, giant cells, Reed Sternberg cells were also recorded. Malignant or suspicious cells were examined more thoroughly if needed under oil immersion objective to get the nuclear details. All the characteristics were recorded before coming to the final conclusion. When malignancy was diagnosed a trial was made to know the grades of differentiation.

Frozen section :

Sections were obtained by cryostat microtome at 4-6 micron thickness at the (-) 30° centegrate. Sections thus obtained were stained with haematoxylin and eosin stain and mounted in D.P.X.

Paraffin section :

The remaining half of the lymphnode was kept in buffered formal saline, further sections from representative site about 0.5 mm in thickness was taken and

was processed in Autotechnicon. Paraffin wax blocks were prepared and sections ranging from 4-5 micron in thickness was taken by microtome, then staining were done with routine haematoxyline and eosin stain and special staining was also done by reticulin, PAS and Zeil-Nelson staining for acid fast bacilli.

The results of impring cytodiagnosis were compared with that of frozen section technique and paraffin section technique to evaluate the accuracy of imprint cytodiagnosis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL ASPECT

The famous Indian Physician, Susruta in the ancient time and the famous Greek Physician Hippocrates (5th Century BC) who has been named as the father of Medicine, had described about the lymphatic system in the human body.

Sushruta has designated lymph to be rasa containing the digested and assimitated food which plays the fundamental role of human nutrition. Hippocrates designated lymph to be white blood or vital requirements of the life. Aristotle had given the description of lymphatic system of the human body. Gasparo Asellius (1622) gave the description of the lymphatic cords distributed in the human body as "exeedingly thin and beautifully white". The description of the thoracic duct and the Cisterna Chyli was given for the first time by Jean Peacquet in 1647. Vanhorne (1652) described the lymphatic system more clearly. In the same year Thomas Bartholin used the term lymphatic for the first time. Rudback (1653) gave the more clear description of the lymphatic system. Nuck (1692), William Hunter (18th Century), Von Recklinghaunsen (1862) have contributed in describing the lymphatic system of human body with its anatomy and physiology to a great extent.

Crusank et al (1786) exhibited the correct and clear chart of symphatic vessels along with its nodes distributed through out the human body. They also described the presence of lymphatics in the skin which become red following infection. Virchow (1863) for the first time described the function of the lymphatic system and stated that the lymphnodes were the barrier preventing the spread of the disease.

DEVELOPMENT OF LYMPH NODE

Robbins (1967) narrated that the lymph node arose in the 3rd foetal month as a local thickening of the mesenchyme along the lymphatic channel. They sent out growth into lymphoid tissue forming the sinuses. The other components of the lymph node were derived either directly or indirectly from the mesenchym possibly through the intermediation of the reticuloendothelial cells that line the premitive lymphatic sinus.

LYMPH NODES

Lymph nodes are small oval or been shaped body. It is the collection of the lymphoid tissue enclosed in a connective tissue capsule lying along the lymphatic system. In the node there is an indented region called the hilus through which blood vessels enters and leave the lymph node.

Lymphatic vessels :

Different vessels : Multiple different vessels pierce at multiple points on the convex periphery of the node and open into the sinus system into the cortex.

Microscopic Anatomy :

The lymph node consists of basically of a lymphoid tissue traversed by special blood vessels or sinuses. The continuous frame work includes a capsule, trabeculae and reticular tissue and the cells entangled in the frame work.

The Capsule and Trabeculae :

The capsule is composed of collagen fibres a few fibroblasts and some elastie fibres specially in the deeper layer. The capsule covers the outer side of the node where they are continuous with the fine reticulam which forms the supposting framework for the lymphoid tissue. At the hilus dense fibrous tissue extend some distance into the medulla. Here the efferent lymphatic vessels are embeded before it kave the nodes. Capillary flexus particularly dense round the lymphatic follicles of the cortex.

The brabeculae are prominent in large lymph nodes but thin and frequently interrupted in small lymph nodes. The hilus in same cases may be absent or slightly

developed. Histologically the lymph node is divided into outer cortex and inner medulla.

Cortex :

Lymphoid tissues though remain scattered through out the node yet found in collected island in the cortex called lymphoid follicles or lymphoid nodules. The centre of which takes lighter stain called germinal centre surrounding thus lighter zone there is a wider area packed with lymphocytes. In the germinal centre new lymphocytes are being formed by active cell division. Numerous macrophages are found in the germinal centre under certain pathological condition and for this reason this central zone is called "reaction centre".

Medulla :

It consists of scattered lymph cells, different varieties of reticulo-endothelial cells and some times few multinucleated giant cells. It is devoid of lymphatic nodules. The cytological arrangement of the trabeculae in the medulla is irregular and frequently anastomosing, lymph cords of it run through the communicating division surrounded by the mashes of trabeculae and lymph cord.

Reticulam :

These tissues extends as a delicate spidary mesh work through out the node. Its fibre bland with the collageneous fibres of the capsule and the trabeculae. It consists of fine reticulam fibres around cells giving a mechanical supports to the cell masses.

Lymph Sinuses :

Every nodes contains tortuous system of irregular channels called sinuses. Such channels both widen and slow the lymphatic flow. Lymph sinuses differ from lymphatic vessels in structure. They are not lined with endothelion system has got three parts.

(i) Marginal sinus underlie the capsule. The afferent lymphatic vessels open into it.

(ii) Cortical sinuses lie between the cortical trabeculae and nodule.

(iii) Medullary sinuses : They lie between the medullary trabeculae and medullary cords. They are confluent at the hilum with the efferent lymphatic vessels draining the node.

Lymphocytic cells :

Lymphocytic cells are mainly of two varieties, e.g., B. Lymphocytes and T. Lymphocytes. B. lymphocytes are located in the superficial cortex forming lymphoid

follicles. They are pale staining, central areas called germinal centres. T. cells are found in the paracortical areas i.e. in between the follicles and deep cortex of the lymph node.

Non lymphocytic cell type in the lymph node :

Steinman et al (1974) describe other cells in the lymph nodes are as follows (i) Endothelial cell, (ii) Fibroblast (iii) Macrophages (iv) Pericascular cells including pericytes and (v) Dentritic cells.

Regenerative ability of lymphnodes:

Young animals can replace excised glands from local tissues. This capacity is lost with the advancement of the age. Local injury to the nodes usually healed by the differentiation of scar tissue.

Involution :

After puberty cortex of the lymph nodes tend to decreases progressively. This decline is associated with regeression of the germinal centre. Eventually the medulla in some cases may even reach the marginal sinuses in some places.

Physiology

Function of the lymph node is divided broadly into the following :-

i) They produce and supply lymphocytes to the blood

and as a supportive function the trabeculae carry blood vessels which supply the nodes.

ii) They screen the lymph by mean of phagocytic activity.

iii) They temporarily stop the spread of cancer cells as these cells have to pass through the lymph vessels to the lymph nodes from cohere they spread in the body.

iv) They serve a great defence role against bacterial infection.

v) They act as mechanical filters to resist the entrance of poisonous substances into the circulation.

vi) They carry out immunological responses. They help in the eleboration of antibodies and in the development of immunity.

The details of the main function

1. Lymphopoiesis :

It is probable that the lymphocytes in the process of malnutration go through repeated mitosis and by further differentiation some will form small lymphocytes and some will be plasma cells.

2. Extramedullary haemopoiesis :

Lymph nodes may be erythropoietic in the late foetal period till birth. In same abnormal circumstance lymph node along with spleen liver and other reticular connective tissue may become erythropoietic and even myelopoietic. This occur in severe anaemia of child hood and in infants with aplastic bone marrow.

3. Filtration and phagocytosis :

The spread of infection and other agents is done by active and intensive filtration in the lymph node. Drinker et al (1934) has demonstrated the filtering pocket of lymph node in a series of experiments. The parenchyma and the reticular mesh work of the sinuses perform the infiltration work.

4. Immunological function :

Mc Master and Rudback (1935) showed evidence that lymphatic tissues produced antibody.

Harris et al (1945) stated that lymphocytes form an indispensible component of the body's immune response.

Parrot et al (1966) there are two population of lymphocytes - T. Lymphocytes (thymus dependent) and B. Lymphocytes (Bursa dependent) which constitutes the thymus dependent and thymus independent areas of lymph node.

REVIEW ON DIFFERENT TYPES OF LYMPHADENITIS

Review on the pathological description of different types of lymphadenitis :

Acute lymphadenitis :

It is caused by virutent bacterial organism like staphylococcus, streptococcus, those get stuck up in the course of its flow in the lymph nodes during its physiological process of infiltration leading to the painful and tender enlarge lymph nodes.

Microscopic feature :

The sinus is crowded with polymorpho-nuclear leukocytes and patches of necrosis which even-tually is converted into an abscess (Boyd 1964) Lymphoid follicle become prominent. There are mitotic figure in the germinal centres.

CHRONIC NON SPECIFIC LYMPHADENITIS

Boyd (1964) stated that chronic lymph-adenitis is a very common condition. The cervical group is most commonly involved due to infection from the mouth, tonsils and teeth.

Macroscope feature :

Glands are firm homogenous having well preserved architecture and moderate enlargement.

Microscopic :

Glands are enlarged and having well preserved architecture. The change is proliferative rather than exudative Reticuloendothelial cells shows gross hyperplasia which become swollen and rounded. Lymph sinuses are greatly dilated called "SINUS CATARRH". Different auther have given the statistical incidence of chronic lymphadenitis. Most of them have stated it to be fairly common incidence.

CHRONIC SPECIFIC LYMPHADENITIS

Tubercular lymphadenitis :

The old name of tuberculosis, 'Scrofula' derived from the latin ward 'SCROFA' which mean large neck of animals. Patients suffering from cervical tubercular lymphadenopathy with large neck was compared to the large neck of the swine in 5th century A.D. Lester (1959). We also find the reference of tuberculosis in "Charaka Samhita" described as one of the most dangerous disease by the physician of the anicient India.

Aetiology :

Mycobacterium tuberculosis of generally speaking acid fast bacilus is the caustive organism of tuberculosis.

GROSS :

Enlarged, matted lymph nodes, firm to soft consistency, cut surface show caseous necrosis appearing as greyish white cheasy like material and some times extensive necrosis may forming cystic space cavity.

Microscopic appearance of tubercular lymphadenopathies

Cytological appearance :

Necrotic tissue i.e. caseous material with epitheloid cell is diagnostic "Sometimes Langhan's type of foreign body giant cell is also found by which the diagnosis becomes more specific.

Histopathological appearance :

In the early stage there is the picture of typical tubercle. There may be central mass of caseation. This is surrounded by histiocytes which are transformed to epitheloid cell. Typically present are the Langhan's type of foreign body giant cell with the number of nuclei often 20 or more which are found at the periphery of the cell. This is further surrounded by lymphocytes and fibroblasts.

SYPHILIS

After the advent of modern chemotherapy the incidence of syphilis has reduced to a great extent.

In primary syphilis the regional lymph nodes are enlarged.

In secondary syphilis the lymph nodes through out the body are affected.

In tertiary syphilis the different systems of the body are affected. The associated lymph node involvement is the rarest or nil. The lymph nodes are painless, discrete, and firm and not fixed to the surrounded structures.

Microscopic appearance :

Proliferation of mononuclear cells lymphocytes and plasma cells. The nodes may be swarmed with spirochetes.

SECONDARY METASTATIC LYMPH NODES

Lymph node cells initiate and activate the tumour immunity which is one of the most important function of the lymph node in the body.

Weiss et al (1980) and Bernhard et al (1957) wrote that lymph nodes are important and indispensible sentinels in immune mechanism. A carcinoma from the primary site first guarded by the arrest of its cells in the regional lymph nodes.

Willi's (1960) and Cole et al (1961) stated that the difference in spread is mentioned in definitive works on spread of tumours. There are two ways by which a

tumor cell reach the regional lymph nodes (i) by embolism and (ii) by its continuous growth along the lymphatics. The cancer cell gradually involves peripheral lymph sinuses, medullary sinuses then destroy the whole lymph nodes by converting it to a mass of cancer. From here it breaks through the capsule to invade the adjacent structures causing adhesion (Boyd 1970). Finally it destroys the overlying fascia and skin giving a fungating mass which also may be ulcerated.

Microscopic appearance :

About the histological classification Custer (1948) opines that a rigid sub classification of lymphatic tumors is artificial and confusing. He showed that there is striking fluidity in histological pattern with transitions and combinations that could best be interpretated as indicating single neoplastic entity having a number of variants.

Whether epidermoid anaplastic or adenocarcinoma its original structure is always reproduced in the metatasised lymph nodes. Thatcher et al (1980) found out that majority of the carcinoma retain their well differentiated feature in the initial stage of the metastasis in the nearest group of the lymph nodes. Very often the secondary lymph node may be more typical and characteristic than the primary.

Immunologically competent lymph nodes show their immune response by reactive follicular hyperplasia, sinus histiocytosis, diffuse lymphocytic infiltration, fibrotic proliferation and cytotoxicity is evident of the process to arrest the growth in the metastatic carcinomatous lymph nodes.

Angiofollicular lymphnode hyperplasia :

In 1954 Castleman, Iverson and Menendez describe 13 cases of localised lymphoid lesion of the mediastinum. The author stressed the benign behaviour of these lesion and regard them as hyperplasia. In 1962 Lattes and Pachter reported 12 new cases, of which only four were mediastinal. Anagnostou and Harrison, 1972 and Keller, Hochholzer and Castleman (1972) have also studied in a series of 81 cases.

Macroscopically the lesion are usually ovoid with a smooth capsule and vary widely in size, often upto 10 cms in diameter or even bigger. They have the colour and texture of lymphoid tissue and on cutting across may show a nodular surface like that of a follicular lymphoma. Microscopically the tissue is made up of an aggregation of lymphoid nodules each about 400 um diameter. In the centre of the lymphoid nodules are collection of pale cells smaller than the germinal centre of true lymphoid follicles. The remainder of nodules are

composed of packed small lymphocytes, sometime arranged in concentric rows like strings of beads. Within the follicles are thicked walled arlerioles that may be radial or parallel to the circumference. Sometime these areterioles are hyline. Increased number of similar arterioles are seen between the lymphoid nodules. The pale centre within the nodules consist of an arleriole surrounded by a cluster of histiocytes. These do not show phagocytosis.

In reticulin preparation the nodules have scanty fibre amongst which the thick walled blood vessel stand out prominantly. Between the nodules there is a regular reticulin meshwork like that of a normal lymph node but with numerous thick arterioles and no visible sinuses.

Benign sinus histiocytosis :

In 1969 Rosai and Dorfman described four patient with enlarged carcical nodes that resembled a malignant histocytosis, microscopically but resolved spontaneously. In 1972 Lennert et al recorded a further case, gave a detail description.

Morphology : Where size has been recorded it has been of the order of 2 to 4 cm diameter in the individual node, but a number of nodes in a group being affected has caused a larger palpable or visible mass. The indivi dual nodes have fibrosis around them. Their cut surface

is bright yellow. Microscopically the capsule is 0.25 to 1 mm thick and continuous with thickened fibrous trabeculae. The severity of the fibrosis appears to increase with the duration of lesion. Lymphoid follicles are present but have scanty in same cases. Reaction centre can be found in them even in older patient. In the least severely affected nodes the basis architecture is preserved and peripheral and deep sinuses are distended with cells, the latter more than the former. In more affected cases the distended sinuses become continuous, isolated the lymphoid tissue into narrow strands. In more severely affected nodes and often in a severely affected part of node the normal structure is destroyed and in these part the reticulum fibres are closely packed, coarse and in an irregular shape.

The cells that pack the sinuses or over run the node are large, pale histiocytes with a diameter of 15 to 25 um. The cytoplasm is usually pale staining, the nucleus has an open chromatin net and usually a large, eosinophilic nucleolous. The characteristic feature of these cells is cytophagocytosis. They contain ingested lymphocytes and some time plasma cells or neutrophil, but erythrophagocytosis is scanty or absent. A second form of histiocytes is of similar size but has an acidophilic ground glass cytoplasm, may have two or more nuclei

and shows little or no phagocytosis. Mitosis are absent or very scanty. In the cords of lymphoid tissue between the sinuses plasma cells and their precursor are numerous and may form the grater part of cell population, plasma cell and lymphocytes are also scar free in the sinuses. Small amount of lipid or glycogen can be demonstrated in the large histiocytes.

LYMPHOMA

The lymphoma according to its cells origin has been divided into :

1. Hodgkin's disease
2. Non Hodgkin's lymphoma

Hodgkin's disease :

Ninety per cent of it originates in the lymphnodes. It originates from the dendritic cells found in the inter follicular region of the lymph nodes.

Non Hodgkin's Lymphoma :

Sixty per cent of Non Hodgkin's lymphomas arise from the lymph nodes. All of them either arise from monoclonal population of B cell or they have no distinctive surface marker. Though the morphological similarity of all the malignant lymphoma are same yet a very few of them also arise from tissue histocytes. Ultman et al (1984). Immunologically the lymphomas may be T. Cell, B. Cell or nuli cell variety.

CLASSIFICATION OF LYMPHOMA

Different authors have given different classification of lymphoma. Some are given below.

Classification by Gall and Mallory(1942)

1. Stem cell lymphoma
2. Clasmatocytic lymphoma } Reticulum cell
3. Lymphoplastic lymphoma } sarcoma
4. Lymphocytic lymphoma
5. Hodgkin's disease
6. Hodgkin's sarcoma
7. Follicular lymphoma

The above classification was based on cell type and degree of differentiation. It was broadly classified as follows :-

1. Hodgkin's disease
2. Lympho sarcoma
3. Reticulum cell sarcoma

CLASSIFICATION BY JACKSON AND PARKER (1947)

1. Lymphocytoma
2. Lymphoblastoma
3. Lymphosarcoma
4. Reticulum cell sarcoma
5. Giant follicular lymphoma
6. Hodgkin's disease
   a) Paragranuloma
   b) Granuloma
   c) Sarcoma

Robbins et al (1984) within the broad group malignant lymphomas segregated Hodgkin's disease (Hodgkin's lymphoma) from all other forms which constitute the Non Hodgkin's lymphoma.

Non Hodgkin's Lymphoma

Rappaport classification (proposed in 1966 and subsequently modified in 1978).

Robbins et al (1984). This is based on two criteria.

1. Cytological characteristic of the lymphomatous cells.
2. Related to two growth pattern
   a) Nodular form :- Here the cells are clustered into identifiable pattern of nodules within the lymph node.
   b) Diffuse form :- Here the cells diffusely infiltrate the entire lymph node without any definite organised pattern.

Diffuse lymphoma (Classification)

1. Lymphocytic well differentiated
2. Lymphocytic poorly differentiated
3. Lymphoblastic
4. Histiocytic
5. Mixed lymphocytic - histiocytic
6. Undifferentiated (Burkitt's and non Burkitt's)

Nodular lymphoma

1. Lymphocytic poorly differentiated
2. Histiocytic
3. Mixed histiocytic - lymphocytic

Lukes and Collins classification (1973)

This is an immunological classification based on the origin of the cells. B. cells, T. cells or histiocytes or mitogen stimulation of lymphocytes(Robbins, 1984).

Lukes and Classification (1973)

(A) B Cell

1. Small lymphocytes
2. Follicular cell. Center cell
   (a) Small cleaved
   (b) Large cleaved
   (c) Small non cleaved
   (d) Large non cleaved
3. Immunoblastic sarcoma (B)
4. Plasmacytoid lymphocytic lymphoma

(B) T CELL

1. Small lymphocyte (T)
2. Convoluted lymphocyte
3. Cutaneous T. cell lymphomas (Sezary syndrome and Mycosis fungoides).
4. Immunoblastic sarcoma (T)

(C) Histiocytic

(D) U (Undefined) cell.

Ultman et al (1984) found out that it is the Rappaport classification which is generally employed as it is reproductable and useful in predicting prognosis.

Microscopic appearance : Robbins et al (1984)

Well-Differentiated lymphocytic lymphoma :

It occurs only in diffuse form. The cell type consists of compact, small, apparent by unstimulated lymphocyte with dark staining round nuclei, scanty cytoplasm little variation in size mitotic figures are very rate. Little or no cytologic atypia. It may contain plasma cells.

Poorly Differentiated Lymphocytic Lymphoma :

Found in both nodular and diffuse type cells are larger. Nucleus is irregular and idented. Chromatin is coarse and condensed Mitosis is rare.

Histiocytic Lymphoma :

The tumour cells are two to three times larger than the normal lymphocytes. Nucleus is larger, vasicular may be round or irregular with marked identation and lobulation. Prominent nucleoli always present. Though it occurs both in nodular and diffuse type the diffuse variety is the commonest.

Mixed Lymphocytic Histiocytic Lymphoma :

It is commonly seen in the nodular form. As the name implies mixed cell population found.

Undifferentiated Non Burkitt's Lymphoma :

The size of the nucleus is same with that of Burkitt's lymphoma but show greater variation both in shape and size. The nuclear chromatin is delicate with single prominent eosinophilic nucleous. The cytoplasm is pale and scanty. Occasionaly multinucleated cells are seen.

About the age incidence the Non Hodgkin's lymphoma may be found at any age. Wintrope et al(1981) and Ultman et al (1984). There are two peak incidence (i) In children and (ii) from 50 years on ward - Desai et al (1965).

Cytological picture of Non Hodgkin's Lymphoma :

Lymphocytic well differentiated type : It reveals immature lymphocytes (Stem cell and lymphoblast) and varried cellular atypia.

Poorly differentiated type :

It reveals lymphoblast and stem cell. Differentiation to lymphocyte is almost lacking. Cell show cytoplasmic basophilia.

Histiocytic lymphoma (Reticulum cell sarcoma).

It shows the presence of lymphocyte, lymphoblasts and stem cell. They can be distinguished from lymphocytic lymphoma by the presence of cells believed to originate from histiocytes (Reticulum cells). These are often larger than stem cells. They have ovoid or irregular nuclei with evenly distributed chromatin and often multiple nucleoli. The amount of cytoplasm is variable. Naked nuclei are very common and are vasicular.

Mixed Histiocytic Lymphocytic Lymphoma :

These neoplasms show lymphoid cells and malignant histiocytes. Malignant histiocytes have pronounced nuclear pleomorphism and often multiple nucleoli (Lukes-1967, Rappaport - 1966).

Plasma cell sarcoma :

Aspiration cytology yeilds atypical plasma cells which readily give the diagnosis. When differentiation is poor the distinction from poorly differentiated lymphocytic lymphoma arising from basophilic stem cells may be difficult. Abundance of cytoplasm eccentrically placed nuclei and presence of binucleated tumor cells may help to establish the diagnosis.

HODGKIN'S DISEASE

Thomas Hodgkin's (1832), a physician of Guy's Hospital, gave more elaborate and informative description of this disease for which another physician, Samuel Wilk (1856) pleaded the name "Hodgkin's disease" for this condition in the memory of the great contribution by Thomas Hodgkin's.

Langhan (1872) described the giant cell in the Hodgkin's disease while studying its histology.

Sternberg (1898) and Dorothy Reed (1902) described the giant cell in the lymphoma with owl eye appearance hence the name of this giant cell, in the memory of these two workers, have been described as Reed-Sternberg giant cell.

Robbins et al (1984) pointed out that Hodgkin's disease has been segregated fron Non Hodgkin's lymphoma for many reasons. Firstly it is characterised histologically by the presence of neoplastic giant cell called Reed Sternberg giant cell admixed with variable inflammatory compound. Secondly, its spread is almost always by contiguity from one chain of the nodes to the adjacent group, thirdly, almost never has a leukaemic component.

It is unchecked there is dissemination to more distanct nodes to the Liver, Spleen and bone marrow.

Lung and C.N.S. involvement is uncommon. It primarily spreads by lymphatics and secondarily by blood stream. In the cervical region it is manifested by painless enlargement of the lymphnodes with few or no general symptoms. Secondary infection become increasingly frequent and the terminal picture is associated with generalised disseminated unbridled malignant neoplasm.

Macroscopically it is rubbery in consistency and pink grey in colour.

Classification of Hodgkin's disease :

Robbins (1984) summarised that unlike situations with Non Hodgkin's lymphoma there is nearly universal acceptance of a single well characterised classification the Rye.

Four distinct pattern has been identified.

1. Lymphocytic predominance
2. Mixed cellularity
3. Lymphocytic depletion
4. Nodular sclerosis

Lymphocytic predominance :

It is characterised by diffuse some times vaguely nodular infiltrate of mature lymphocytes admixed with variable number of benign histiocytes. Scattered amongst these cells are distinctive RS Cell (Robbins 1984).

Mixed Cellularity:

It is marked by a diffuse infiltrate of lymphocytes, histiocytes eosinophilis and plasma cells. RS cell are usually plentiful. Number of lymphocytes are less (Robbins 1984).

Lymphocytic depletion :

It shows a paucity of lymphacytes and relative abundance of RS cells or their atypical pleomorphic varient (Robbins, 1984).

Nodular sclerosis :

It is only one which is more common in woman. There is a tendency of the cells to assume the lacunar morphology. Classic RS Cells are infrequent (Robbins, 1984).

Cytology in Hodgkin's disease :

Lymphocytic predominance is usually associated with the early ages. Nodular sclerosis and mixed cellularity is usually distributed in both the early and advanced ages. Lymphocytic depletion is commonest in advanced ages (Lukes et al 1966).

This histologic grouping tallies to some extent with the finding in smears of biopsy aspirates. The first group is dominated by lymphocytes but they also contain numerous histiocytes some of which are enlarged to have

lobulated nucleus but not prominent nucleoli. Hodgkin's giant cells may also be found. These resemble basophilic stem cells but are much larger and contain prominent round nucleous. A finding of large histiocytes and Hodgkin's cells possibly with some eosinophilic granules should arouse the suspicion of Hodgkin's disease however the identification of Reed Sternberg giant cell having segmented or multiple nuclei similar to those of bone marrow megakaryocytes but containing large nucleoli that may attain the size of erythocytes.

In nodular sclerosis type of Hodgkin's disease biopsy aspirate are selectively taken from cellular areas of lymph nodes. The smears can not be distinguished from those in the mixed cellularity type.

In Hodgkin's disease with mixed cellularity aspiration biopsy usually yeilds abundant material. The cell pattern shows Reed Sternberg cell in addition to lymphocyte histiocytes, eosinophilic cells plasma cells.

Lymphocytic depletion is the common feature of several histologic expression of Hodgkin's disease. Some cases show diffuse fibrosis others are described as in aspiration biopsy shows few fibroblast like cells and giant cell with nucleoli of malignant appearance.

Histological appearance :

Pleomorphism is the most characteristic feature of the microscopic appearance of Hodgkin's disease. The eseential change is the proliferation of RE cells which gradually replace the lymphocytes until one of the latter may be seen. The new cells are large pale with vasicular nuclei.

The Reed Sternberg giant cells are 15 to 40 micrometer in diameter, the cytoplasm being plentiful. There are two or more nucleio one of which is mirror image of the other.

There is partial or total obliteration of normal follicular and sinusoidal architecture by a diffuse and frequently mixed infiltration of lymphocytes eosinophilis, histiocytes, plasma cells and neutrophilis. Accompanying Reed Sternberg cells may be either sparse or plantiful (Wintrope, 1981).

Though in absence of Reed Sternberg giant cell diagnosis of Hodgkin's disease should not be mame yet presence of such cells by itself is not pathogenic of the disease (Ultman et al, 1984).

OTHER DISEASES CAUSING LYMPHADENOPATHIES

Leukaemias :

Penington (1984) found that (i) enlargement of the superficial nodes is the outstanding clinical

feature of the leukaemias, (ii) slight to moderate enlargement of the lymph nodes are common in later stages of leukaemias.

Microscopic Picture :

In lymphatic leukaemia the normal structure is replaced by masses of immature lymphocyte and while in myeloid leukaemia by immature myeloid series of cells.

Other diseases causing lymphadenopathy are actinomycosis, acute hepatitis, AIDS, angio immunoblastics, brucellosis, drug induced - Phyenytoin, Mephentoin, Captopril, german measles, infectious mononucleosis, sarcoidosis, systemic lupus erythematosus, rheumatoid arthritis, rheumatoid arthritis and tularemia.

IMPRINT CYTOLOGY

Imprint cytological study as an adjuvent to biopsy diagnosis has been tried for back in (1921) by Tutharie, since then a house of workers have critically evaluated and re-evaluated its diagnostic importance, however, this technique has not received universal attention, it deserves Forkner (1927) is credited to have described the cytology of lymphnode for the first time. Berman (1953) gave an account of histopathological correlation with the imprint cytology of lymphnode and indicated that these are complementary to each other.

Dudgeon and Patrick 1927 introducing wet film method whose maiden work in 200 cases with only 9 errors, still remain the basis of study. It was the work of Dudgeon and Jewesbury in 1924 using Schanudinn's solution for the cytological examination of human milk which prompted the use of cytological diagnosis of tissue.

The touch preparation or imprint cytology has now been widely accepted as the method of choice for cytological diagnosis being a more accurate method than a scrapping (Moore and Reagan 1953 and Ghandur, 1985).

Dudgeon and Patrick (1927) and Dearing (1953) used a smear prepared by scrapping the tissue with a scalpel. Houptman (1948) found both smear and touch preparation unsatisfactory because the cells were unevenly distributed and elongated in the direction of smear and also tissue rich in mucous or fat (e.g. mucosa, the gastrointestinal tract and breast) and fibrous tissue gave smears poor in cells.

Crush method was used by Coley (1931),Martin (1934) and Stewart (1933) in which forceful spreading of tissue was done between two slides. Again, Hauptmann disapproved this method on the basis that too many clumps of cells were present to permit uniform staining or preserve the shape of the cells. He found this method of placing a fragment of tissue not larger then 2-4 mm in

diameter in 3-4 drops of pooled human plasma and teasing apart of the tissue with two fine needles and drawing the suspension between two slides very effectively.

This method was similar to that used by Haematologist in the preparation of blood smears. Despite this controversy the last two decades have been the emergence of touch preparation as the sole survivor (Tribe 1965; Ultmann 1958; Sakai and Lauslathi, 1969; Suen et al, 1978; and Ghandur 1985).

Various stains and staining techniques have been used by cytopathological research workers in an effort to obtain better results. The aims is to preserve cells as well as possible and at the same time to differentiate clearing the various cellular structures.

Dudgeon and Barrette (1934) used Shaudinn's solution and subsequently treated the cells with haematoxyline, Iodine and Eosin. Martin and Ellis (1930), Pickeren and Burke (1963) used haematoxyline and eosin and found this most satisfactory. Hauptmann (1948) studied several preparations some stained with a combination of May Grunwald Giemsa's stain. Others with Wilson's stain (Phosphate buffered wright stain) and still others by Papanicolaou's method (Haematoxylin - EA 25 - OG 6).

Moore and Reagan (1953) used Papanicolaou's EA-50 and found this very useful. Ultmann (1958) compared

Wright-Giemsa and Papanicolaous's stain on lymph node imprints and smears.

Tribe in 1965 used polychromatic methylene blue for rapid staining and May-Grunwald Giemsa stain for routine cases.

Sakai and Lauslathi (1969) employed a modification of Moriquand cytological technique where preparations were fixed in Delaunay's solution for 2 seconds, and subsequently treated with either of three stains viz. haematoxylin, eosin , Papanicolaous EA 50 and Shorr Pundel Trichome stain.

The result with the stain and technique used however only reflects a familiarity with the method and not a superiority of one over the other (Ultmann, 1958).

Diagnostic accuracy of Frozen Section and Imprint Cytology :

Imprint cytology is now a well accepted and a reasonably good method for screening of inflammatory, benign and malignant tumour per operatively with result comparable or even better than frozen section.

Lucas (1955) as described by Miale (1972) with slight modification performed aspiration and imprint cytology and found these was little difference

between two types of smears. Out of which the diagnosis of 52 cases was made by aspiration and found 61% accuracy rate, more over he revealed different cell lineages as reticulam cell 0-0.1%, mast cell 0-0.5%, lymphoblast 0.1-0.9%, Prolymphocytes 5.5-16.4%, lymphocytes 67.8-90%, monoblast 0-0.5%, Promonocytes 0-0.5%, monocytes 0.2-7.4%, Plasmoblast 0-0.1%, proplasmocytes 0-0.5%, Plasma cells 0-4.7%, neutrophils 0-2.2%, eosinophils 0-0.3% and basophils 0-0.2%.

Ultmann et al (1958), In his stidy from 165 specimen of lymphnode 166 lymphnode imprint perform and studied by wright-Giemsa technique, out of which 93 lymphnodes were diagnosed as benign disease and 73 as malignant disease there were 5 false positive (3.0%) and 6 false negative (3.5%) reports with Papanicolaous stain only 116 lymphnode imprint was studied and in which there was 3 false positive (2.6%) and 5 false negative (4.3%) reports.

Ackerman and Ramirej (1959) reviewed 1269 consecutive frozen sections of different organs including 143 frozen sections of lymphnodes and he claimed over all accuracy of diagnosis with frozen section as 98%. However, the diagnosis of lymphnode lesion was cent percent correctly diagnosed. He found total malignant tumour of lymphnode was 63, no benign tumour was found as false positive, error was zero and false negatives were 3 and the over all error was 2%.

Tribe (1965) felt that imprint could be cheaply and reliably used in countries or organisation where there was a lack of trained technician or apparatus needed for forzen section. Masukawa et al 1966 noted that in rare cases where a tissue biopsy can not be obtained, a cytologic examination may yield a diagnosis.

Bloch Max 1967, In their study lymphnode puncture is explained and the material utilized for this study was analysed and their results were divided into inflammatory tuberculous lesion, malignant lesion and miscellaneous. The malignant lesion was further subdivided into Hodgkin's disease, metastatic and lymphosarcoma, reticulin cell sarcoma and leukaemia out of 45 cases, 40 lesions were diagnosed i.e. 89%.

Sakai and Lauslathi (1969) in which studied 400 frozen section preparation 135 cases (33.7%) was malignant lesion and 248 cases (62.0%) was benign. Nine cases (2.3%) was adaquate as doubtful and 8 cases (2.0%) as false negative. Thus the overall accuracy rate by frozen section was 95.7% and in 123 cases of cytologic smear (30.8%) was classified as positive and 16 cases (4.0%) as strongly suspicious, further 10 cases (2.5%) was diagnosed as suspicious 243 cases (60.7%) as negative, the specimen of 3 cases (0.7%) was unsatisfactory. There

was one false positive and 4 false negative cases. Thus the accuracy rate by cytological study was 95.5%. Out of 400 cases only 43 lymph node was negative and 17 are positive. There was no false positive and false negative result and by frozen section diagnosis out of 43 cases 26 was benign and 17 are malignant. There was also no false positive and false negative results.

Sankaran and Reddy (1970) they reviewed 80 cases of imprint smear with histopathological finding 100% correlation was observed with histopathological findings in cases of acture lymphadenitis and lympho-sarcoma, and 69.6 to 60.8% in non neoplastic lesion the smear diagnosis was confirmed by tissue reports in 79% of the cases and also recorded an accuracy of smear diagnosis in 60% of cases of Hodgkin's disease.

Aust et al (1971), In their study of 100 head and neck tumours reported no false positive and malignant and only 2 deferred diagnosis, achieving an accuracy of 98% they too were of the opinion that with experience tissue imprints can replace the more complicated frozen section.

Prasert Tanapatachaiyapong, 1972 studied lymph node imprint cytology after modified technique, preparing lymph node imprints by rolling a glass slide surface on the cut surface of lymphnode and staining with a modified

concentration of wright-Giemsa stain and found excellent reproducibility and maximum cytologic details.

Holaday and Assor (1974) in his study 10,000 consecutive frozen section consultation performed and assess the accuracy of the method and to develop a quality control mechanism. Frozen section interpretation of specimen of lungs, tumour of skin, thyroid, parathyroid, and tissue from the female genital tract were in error is about 1.5% of cases. Specimen from gastrointestinal system, otornionlaryngal region, lymphnode, central nervas system and pancrease were incorrectly interpretate in fewer than 3% of instance. One false positive frozen section in the 842 lymph node examined consisted of reactive hyperplasia (sinus histiocytosis) which was interpreted as metastatic carcinoma and false negative, of which there were five, due to sampling error by both pathologist and the surgeon.

Mathe et al (1975), In a study of imprints from 141 lymphnode reported that cytology did not add more information to histology in 39 cases (27%). It modified or helped histological classification in 67 cases (48%) and it helped to confirm that the suspected diagnosis of reticulosarcoma was in fact metastatic carcinoms in 18 cases (13%).

Godwin (1976) he reviewed that lymphnode submitted for immediate frozen section cytological preparation readily differentiate benign and hyper plastic lymph node from metastatic carcinoma and granuloma often times the node contain so few metastatic cells that the smear preparation provides a better sampling than a frozen section. In addition to the problem of obtaining good frozen section from such matermal.

Agarwal et al (1977), In their study imprint smear prepared from 135 lymphnode was taken including 10 normal ones, of 125 disease lymph nodes and found 85 was inflammatory, 18 primary neoplasm and 22 secondary neoplasm. It was also found that technique was quite reliable and correct diagnosis was made in 97.6% of the case. In 2.4% of cases in whom cytodiagnosis was a failure, 2 cases was of sclerosing type of Hodgkin's disease and one was a case of metastasis from fibrosarcoma in the lymph node.

Bloustein and Silverberg (1977) studied 21 benign lymphnode, 23 with metastatic carcinoma and 4 with lymphoreticular neoplasm. They state that small focus of cancer in a lymphnode can be mixed by frozen section with the conventional method of sampling the node and that accuracy in detecting metastasis in large lymph node is increased by the use of imprints.

Emphasized that imprint obtained prior to frozen section examination was helpful in better evaluation of the forzen section due to its better cytomorphological details.

Suen (1978), In his study out of 1258 cases, 198 lymphnode imprint studied and found 96% correct diagnosis in metastatic carcinoma of lymph node and 88% correct diagnosis of lymphoma, 63% correct diagnosis in granuloma and 99% for reactive and normal lymph node and false negative rate was 5%. To increase diagnostic accuracy be recommened the combined use of imprints and frozen section this observed that the imprint method was useful and could provide valuable information when frozen section interpretation is equivocal particularly in : (1) Diagnosis of neoplastic lesion which simulate inflammatory lesion in frozen section viz lymphnode metastasis.

(2) Benign inflammatory lesion which can simulate malignancy in frozen section, like organizing pneumonia, intense histocytosis in lymphnode.

(3) In the diagnosis of malignancy confined to one small area of large specimen. The amount of tissue that can be frozen for rapid intraoperative diagnosis is limited. On the other hand imprint method can cover a large portion of specimen easily.

(4) In the diagnosis of malignancy when the submitted specimen is limited in quantity.

(5) In the diagnosis of malignant lymphoma.

(6) He recommended the routine use of imprint in conjuction with forzen section for the following region.

(i) It helps in the diagnosis and classification of malignant lymphoma.

(ii) It helps in the diagnosis of metastatic round cell tumour which may simulate lymphoma and

(iii) It reduces sampling errors.

In conclusion he points that imprint cytology and frozen section complement each other in ensuing accuracy in rapid tissue diagnosis and maintain that in certain situation imprint is superior to frozen section methods.

Tyagi et al (1981) studied 136 cases of lymph-adenopathy and evaluated the role of imprint cytology. Smear were stained with 1% toludine blue and haemato-xyline eosin. The result was compared with histopatho-logical results and morphological diagnosis in these cases 92 cases (67.6%) of tuberculous lymphadenitis, 26 cases (19.1%) of non specific lymphadenitis and 8 cases (5.9%) of metastatic lymphadenopathy. The over all accuracy rate was 73.6% in 18 cases (13.2%) the

diagnosis was negative. In cases of malignancy (Primary or secondary) the smear was diagnostic in (100%). The only difficulty arose in case of non specific lymphadenitis and in differentiating between follicular reactive lymphnode and lymphoma.

Nagpal et al 1982 studied 50 cases of lymphadenopathies without any consideration of age and sex and evaluated the role of imprint cytology. The lymph node choosen for study were bisected and half of the lymphnode be fixed in 10% formal saline solution for histopathological examination by paraffin wax technique and from other half 4 to 5 imprint smear was made he represented over all accuracy rate as about 94%. It was 60% in chronic non specific lymphadenitis, 95.4% tuberculous lymphadenitis, and 100% each in lymphoma, Hodgkin's disease and metastatic carcinoma.

Tung-Kwang Lee (1982) emphasize that imprint cytology can some time provide information on the histogenesis and histologic pattern of the tumour, but it can not provide information on the depth of infiltration. Despite that limitation and the percentage of the false negative result that it was yield (8.1%) of malignancy in his study. He also noticed that the imprint technique was reliable and for rapid diagnosis of tumour and recent report indicate that cytologic material for surgical specimen,

submitted for forzen section can be readily diagnostic as the frozen section itself.

Verma Suprabha et al (1983) in their study of 60 patiehts of lymphadenopathy, imprint smear was made and found diagnostic accuracy as 83.3% and advocated these tools as sufficiently reliable, simple quick and economic out patient procedure.

Ronald et al (1983) he carried out his study in 42 patient with localised prostatic cancer undergoing staging pelvic lymph node biopsies. Out of 42 patient 13 (31%) had metastatic disease of lymph node on permanent section. Frozen section diagnosis correctly predicted 10 of these 13 cases, for a falsely negative rate of 7% of (3 cases).

Chaudhary, Major, M. (1984) In their 52 unselected lymph node biopsies, imprint smear and frozen section were taken and their result was compared with the paraffin section diagnosis. He found accuracy as 90.4% and 93% in cases of imprints and frozen section respectively.

Quill et al (1984), in his study, lymphnode imprint cytology was performed on 86 nodes from 13 consecutive with breast cancer undergoing simple mastectomy with axillary node sampling and a prospective comparison with paraffin section was made. The

results showed a diagnostic sensitivity and specificity of 0.93 and 0.98 respectively.

The predictive value of a positive result was 0.98 and also emphasized that technique can be used to identify patient with stage I disease rapidly, there by allowing their exclusion from treatment in the perioperative chemotherapy.

Ghandur's (1985) result of touch preparation of metastatic lymphnodes showed it to be equal or even superior to the conventional frozen section. In his hands the accuracy rate was 98.7 by frozen section and 99% by touch preparation. He recommended that frozen section be replaced by tissue imprints and be used as the sole method for intraoperative diagnosis of lymph node metastatic disease.

Bhalla et al (1986) in their study out of 212 cases, 57 lymphnode were taken and 41 lesion diagnosed as malignant by imprint cytology and paraffin section and 16 lesion diagnose as benign by imprint and paraffin section. Hodgkin's disease was diagnosed in 11, Non Hodgkin's lymphoma in 10, metastatic tumour in 20 and benign lesion in the remaining 16 cases. Total accuracy was 100%.

Ademiluyi et al (1986) In their study 50 consecutive non leukaemic patient were taken with enlarged lymph nodes. The lymph node was exicised, bisected and imprint smear was made. On cytological and histological examination, they opined that in 33 (66%) cases, the degree of correlation between the result of both method was significant. The level of matching of the imprint diagnosis was 100% for Burkitt's lymphoma and 91.67% for Hodgkin's disease and 84.62% for metastatic carcinoma. There was poor matching, however, in Non Hodgkin's disease (40.0%) and in tuberculosis (30.77%).

Kaufman et al (1986) 526 consecutive frozen section of different organ including 79 lymphnode specimen were diagnosed by frozen section. He claimed overall accuracy of diagnosis with frozen section as 97.1%. However the accuracy for lymphnode specimen was 96.2% false positive result in lymphnode was 1.3% and false negative was 2.5%.

Rogers et al (1987) In his study frozen section diagnosis was performed to determine the source and nature of inaccuracies associated with this procedure of 30,278 surgical pathology specimens accessioned 1414 (.47%) had frozen section examination of these, there were five false positive diagnosis of malignancy (0.4%), 16 false negative diagnosis of malignancy (1.1%) and 53 deferred

of diagnosis (3.7%). Soft tissue, breast and lymphnode sites accented for 12 error (57%). Erroneous frozen section diagnosis was atributed to interpretation (57%) microscopic sampling (24%), gross sampling (9.5%) and lack of communication between pathologist and surgeon 9.5%. Some of these diagnostic error might have been divided by changes in procedure or technique.

Stevens et al (1987), Aims of his study was to assess the usefulness of the lymphnode imprint technique in quantitating cell types and comparative study using cytocentrifuge smear as control was undertaken from 12 patient with histologically confirmed non Hodgkin's lymphoma using semi automated image were analysed. These cases were classified in accordance with the modified Rappaport scheme. These comprised 3 cases of diffuse well differentiated lymphocytic lymphoma, one of nodular poorly differentiate lymphocytic lymphoma, two each of nodular and diffuse mixed lymphocytic histiocytic lymphoma, three of diffuse histiocystic lymphoma and one of undifferented lymphoma of non Burkitt's type. The results show that the imprint procedure selective bias in favour of certain cell types, moreover, certain cell measurement, such as nuclear shape was easier to see on cytocentrifuge smear. There were no significant differences in the mean nuclear area measurement between imprint and cytocentrifuge smear. The standard deviation

of nuclear area, obtained on imprint was consistently lower compared with that using centrifuge smear suggesting a less dispersed cell population the systemic error or bias of the measurement was significant ($p < 0.05$).

Gupta et al (1988) studied 188 patient with lymphadenopathy their age ranged from 1 to 70 years with detailed clinical datas and found out of 188 patient, 101 had involvement of cervical, 48 abdominal, 12 inguinal, and 7 axillary groups of lymphnodes. Twenty patients were having generalized lymphadenopathy, histopathologically in their study, commonest cause of lymphadenopathy was found in tuberculosis (40.4%) followed by non specific lymphadenitis (30.9%), metastatic lesion (15.4%), lymphoma and leukaemia (10.1%). In 6 cases (3.2%), no lymph node structure was seen. In patient with generalized lymphadenopathy, the commonest cause was lymphoma, which was observed in 8 out of 20 cases (40%). Imprint cytology confirmed the diagnosis in 70 out of 86 cases studied giving a diagnostic accuracy of 81.39%. The highest percentage of accurate diagnosis by imprint cytology was in cases of tubercular lymphadenitis (88.88%) where only 3 cases was misdiagnosed. In case of chronic non specific lymphadenitis accurate diagnosis was 81.57% were only 7 cases are misdiagnosed and in cases of lymphoma the accurate diagnosis by imprint cytology was 76.92% where 3 cases was misdiagnosed and in cases of metastatic, the accurate diagnosis was 62.50%,the misdiagnosis in 3 cases.

Jina et al (1989) studied 64 cases out of which 14 were lymphnodes lesion and found percentage of correct diagnosis of lymphnode malignancy by imprint cytology was 87.50% with both stain i.e. H & E and Pap. In Hodgkin's disease and metastatic lesion the diagnostic accuracy was 100% at the same time he also noticed that imprint smear of lymphnode has been more informative then aspiration smear. Pap stain had given better results then H & E stain. This is in accordance with the results of the other author (Lucas, 1955; Ultman et al 1958 and Mehrotra et al, 1977).

Anuraradha, S. and Parathasarathy, V.(1989) evaluated imprint cytology and fine needle aspiration cytology of lymphnodes and observed over 100% accuracy in cases of chronic non specific lymphadenitis, tuberculous lymphadenitis, non Hodgkin lymphoma and metastasis but in Hodgkin's lymphoma accuracy was 87.5% and concluded over all accuracy rate as 94% with these technique.

FROZEN SECTION

The earliest utilization of frozen section in clinical diagnosis is attributed to Welch of Johns Hopkins Hospital. Who is said to have employed this method in 1891 on a patient operated upon by halsted for a benign breast tumour. In 1895, a detailed technique for the procedure was outlined by Cullen. Wilson

(1905) adopted the method for use at the Mayo Clinic, and Lockwood (1906), in an address to the Hampstead division of the Poritish Medical Association draw attention to the value of an immediate microscopic examination of tumour and diseased tissue at operation. Shaw (1910) in his communication, pointed out the advantage and a modified method of frozen section, for its wider application.

Since MacCarty's reports of his result with more than 208,000 such examination in 1929, there has been a spurt in its usage. Ewing (1925), Bloodgood(1934), Simpson (1937) and Breuer (1938) have all unequivocally agreed upon utility of frozen section as a rapid diagnosis tool.

The last three decades has been a revival because of two reasons : At first, a number of detailed studies by Ackerman and Rawirez (1959), Winship (1961), Sparkman (1962), Nakazawa et al (1968) and Holaday and Assor (1974) has shown the indication and limitation of frozen section, and the histological evaluation of different tissue has solved many problems which the pathologists faced while solving the frozen section. Secondly the introduction of cryostat has improved the quality of frozen section that is generally obtainable otherwise (Horn 1962). They provided a rapid and

easy means of preparing large thin unwrinkled section of single or multiple pieces of fresh frozen tissues. The sections have a high resolution of cytologic details required for definitive pathologic diagnosis, histochemistry, fluoroscence microscopy and autoradiography.

During its 90 years of existance, the frozen section method is prone to the brunt of much criticism, earliest opposition of the procedure, arose from its technical shortcomings.

Bloodgood (1934), commented that "The greatest danger today is that a diagnosis erroneously made on borderline tumour will lead to an unnecessary radical operation". This was corroborated by Ackerman (1959), who contended that it had to happen two or three times to invalidate completely the use of frozen section diagnosis in that hospital.

He was of the opinion that frozen section should not be used in every case nor pathologist used as technicians to facilitate the surgeons intellectual curiosity.

In 1937, Simpson affirmed that most frozen sections examination, even in cancer, were superfluous since gross examination usually was adequate for diagnosis.

Breuer (1938), admitted that the diagnostic qualities of slide made by frozen and paraffin methods were not significantly different, but confessed that it was necessary for him to revise 11% of this frozen section diagnosis after final study of paraffin section.

He blamed this disparity upon the element of haste which precluded the aliberate and unhurried study of one or multiple sections, and which exerted a disfurtring psychological affects upon the pathologist.

Foraker in an allegory published in 1960 entitled "Co-operation versus conflict in the surgical pathology consultation" considered the surgeon to be equally at fault in demanding categorical diagnosis.

In contrast, at Mayo Clinic a quick frozen section is done in every case for which tumour is removed regardless of its nature, and a report of the findings given to the surgeon within a few minutes (Dockerty, 1953). As a result of this he reports a change in 8% of operative procedure over a period of forty years.

As the procedure has become more common place, criticism of it have assumed a different character. It is now acknowledged that the method is only as efficient as the man who applies it, and that the pathologist who is inept an inexperienced is likely to encounter trouble (Jehning and Larider 1957).

INDICATION

Ackerman and Ramirez (1959), confirm that major indication of frozen section is to reach a therapeutic dicision. Most commonly this involve, the primary diagnosis of the tumour, its nature, the extent of local or distinct spread of malignant tumour and the evaluation of the adequately of resection before the wound is closed. Less frequently frozen section is indicated to determine whether diagnostic tissue has been obtained in a biopsy, although further surgeon is not contemplated at the time. Although exact histologic diagnosis is desired, in most instances it is sufficient to know that the tissue present is a malignant tumour or non neoplastic.

Horn (1962) too agrees that attempt at frozen section diagnosis may be worth while where a decision as to the malignancy or a benigness of a disease process has any change of influencing the surgeon in dividing upon his immediate cause of action.

LIMITATIONS

In his 40 years experience at Mayo Clinic, Dockerty (1953), had achieved following objectives:

1. Rapidity not more than 60 secs being required to prepare, cut, mount and stain a section from the average block of tissue.

2. Uniformity and thinness of the section to a tolerance which permits of their being routinely examined cytologically with the oil immersion diagnosis to the microscope.

3. Universal application to sectioning of practically all solid and semisolid tissue of the body (bones and teeth are exception to the rule).

4. Elimination of all preliminary treatment of tissue such as farmalin fixation boiling, drying, or other dilatory hocus-pocus.

In 1969, Sakai found frozen section to the advantageous in detecting the type of tumour and its invasive properties, but failed to achieve the high power microscopic details of paraffin section.

Tribes (1965), on the basis of his study, found frozen section to be inferior preparation as compared with its paraffin counterpart because it was usually thicker and lacked the familiar artifacts produced by dehydration, he however submitted that cryostats had bviated many of the disadvantages.

A number of workers have stressed that to achieve a high accuracy rate, adequate clinical back ground data is very important.

Ackerman (1959) has properly noted that this should include the oppourtunity to study pathologic material that might have been obtained at previous surgical procedures, and the gross examination of the speciman or lesion either in the lab or in situ, including careful selection of the most suitable portion for histological examination.

He and Nakazawa (1968) both emphasise the need for frozen section being performed in the operating suite where the pathologists and surgeon have more opportunity to discuss the particular case under consideration.

Nichalas, as far back in 1927, opined that frozen section not only furnishes a rapid means of diagnosis but also brings in close contact the pathologists and clinician, whose mutual interest in the case is thus greatly increased from both scientific and practical stand point, the ultimate beneficiary being the patient. French (1960), on the other hand, does not think this necessary and considered it advantageous to have the specimen sent to the department of pathology where adequate technical help and diagnostic acumen of all members of the department are brought to bear upon a diagnosis.

Sparkman (1962), found the following factor responsible for errors in diagnosis :

(a) Unusal lesion

(b) Short comings of the method

(c) Short coming of the surgeon

(d) Short coming of the pathologist.

It is recognised that certain tissue tend themselves poorly to examination by the conventional frozen section technique.

Nakajawa et al (1968) preferred to delay diagnosis in cases of papillary tumour of breast, primary tumour of soft tissue and lymphnodes, suspected of imvolvement by lymphoma.

Ackerman added to this list, inflammatory cells trapped by dense connective tissue and scar, proliterating psedomucin formed by mesothelium endometrium within lymph nodes.

Horn (1962) and Nakazawa (1968) pointed out that small biopsies which nibble at the edge without being carefully chosen may prolong the term required to accurate at diagnosis. They contend that an adequate and representative tissue must be made available for evaluation and this should generally included material for an portion to be preserved for preparation of permanent section.

The principal contradiction to the performance of frozen section examination is the risk of implantation

in tumour of lung, thyroid, stomach and soft tissue sarcoma (Ackerman 1959, Horn 1962).

Holaday and Assor (1974) and Sparkman (1962) opined that because early and borderline lesions are being examined more and more frequently, many specimen are submitted which are difficult to interpret, however, the slides are prepared. In equivocal situation many workers emphasize to defer diagnosis or to err in the benign rather than malignant direction. Ackerman states that there are only three possible diagnosis; positive for cancer, negative for cancer or no diagnosis made. Jehnings and Lander (1957) state that such a pharse as "almost certainly malignant" has no place in the language of frozen section diagnosis.

TECHNIQUE

A number of suitable methods have been detailed usually as modification, minor or otherwise, if one or another method.

Dockerty in (1953) used a spencer freezing microtome with a blade angle of 30° with liquid carbon dioxide and neutral polychrome methylene blue staining. He further stressed that the average block should be rectangular and its dimension should not exceed 1.5 x 1 cms on surface of about 5 mm in thickness.

Murray et al (1959) used a chilled knife microtome and an altered Haematoxylin and Eosin stain. Although the conventional rotary and freezing microtomes have been in use

for over half centuary with effective results; their efficiency is greatly limited by the condensation of water vapour if the atmosphere is warm or humid (Klionsky et al (1960).

The Linder strom lang cold chamber cryostate was introduced in 1938 for preparation of frozen tissue histochemical research. The utilisation of this instrument in the examination of surgical tissue was first reported by Russell, Chang and associates in 1960. The cryostate consists essentially of a cold chamber, cooled by mechanical refrigerator containing a microtome. Although this method requires some what more time than conventional technique. The preparation is permanent cytologic detail is well preserved for examination under the high dry or oil immersion objectives. Section of single or multiple pieces of fresh frozen tissue can be cut and mounted. The resultant sections are large for ease in orientation and are thin, unwrinkled and undistorted in cytologic detail. The principle advantage of the cryostate lies in the fact that many tissues which are unsuitable for preparation by the customary frozen techniques are readily sectioned with the instrument viz. small fragment including breast papilloma, breast tissue, and aspiration biopsies. Other tissue generally regarded as difficult to freeze including thyroid endometrium lymph node and lungs yield section suitable for diagnostic study (Sparkman 1962).

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

In the present study 114 cases of lymphadenopathy were studied. The age of these cases ranging from 2 to 70 years and were of either sex. The involved lymph nodes were of cervical, axillary, inguinal and or mesentric group or showing generalised lymphadenopathy.

TABLE - I : Showing distribution of various cases of lymphadenopathy according to site.

Groups of lymphnodes	Regional distribution of lymphadenopathy	
	Number of cases	Percentage
Cervical	45	39.5
Axillary	31	27.2
Mesentric	11	9.6
Inguinal	06	5.3
Generalised	21	18.4
Total	114	100.0

Table - I shows the involvement of various groups of lymphnodes. Out of the 114 cases, cervical lymphnodes were involved in 45 (39.5%), axillary in 31 (27.2%), mesentric lymphnodes in 11 (9.6%), inguinal lymphnodes in 6 (5.3%) cases and generalised lymphadenopathy was seen in 21 (18.4%) cases.

TABLE - II : Correlation of age with sex in cases of lymphadenopathy.

Age group in year	Total No.of cases	Per-centage	Age-Sex distribution of lymphadenopathy			
			MALE		FEMALE	
			No.of cases	Percen-tage	No. of cases	Percen-tage
0 - 10	14	12.3	9	7.9	5	4.4
11 - 20	25	21.9	9	7.9	16	14.0
21 - 30	25	21.9	6	5.3	19	16.7
31 - 40	16	14.0	7	6.2	9	7.9
41 - 50	14	12.3	4	3.5	10	8.7
51 - 60	15	13.2	8	7.0	7	6.2
61 - 70	05	4.4	2	1.7	3	2.6
Total	114	100.0	45	39.5	69	60.5

As regards the age of the patients studied (Table-II) 14 (12.3%) patients belonged to age group of 0 - 10 years, 25 (21.9%) in each of 11-20 years and 21-30 years, 16 (14.0%) in 31-40 years, 14 (12.3%) in 41-50 years, 15 (13.2%) in 51-60 years and 5 (4.4%) in 61-70 years. Out of 114 patients 45 (39.5%) were males and 69 (60.5%) were females. So maximum number of cases (50) were belonging to young adults (from 11-30 years age).

TABLE - III : Lymphadenopathy in relation to regional involvement.

Groups of lymphnodes	Total No.of cases	Chronic lymph-adenitis	Tuber-culous lymph-adenitis	Reactive hyper-plasia	Hodg-kin's lym-phoma	Non Hodg kin's lym-phoma	Meta-sta-sis	Misce-llane-ous
Cervical	45	5	24	4	-	5	6	1
Axillary	31	3	15	2	-	2	9	-
Mesentric	11	2	3	3	-	2	1	-
Inguinal	6	2	3	-	-	-	1	-
Generalised	21	1	8	-	6	4	1	1
Total	114	13	53	9	6	13	18	2
Percentage	100	11.4	46.5	7.9	5.3	11.4	15.8	1.7

In cervical lymphadenopathy (Table-III) maximum number of cases (24/25) were of tuberculous lymphadenitis, axillary lymphadenopathy and inguinal lymphadenopathy were also mainly due to tuberculosis axillary lymphadenopathy 15/31, and inguinal lymphadenopathy 3/6. While in generalised lymphadenopathy incidence of tuberculous lymphadenitis was 8/21 and in mesentric lymphondes were of 3/11 cases. Reactive hyperplasia was observed in 3 out of 11 cases of mesentric lymphadenopathy. Most of the cases of generalised lymphadenopathy were of lymphoma (10/21), Hodgkin's lymphoma and non Hodgkin's lymphoma). One case of generalised lymphadenopathy was showing benign sinus histiocytosis. Metastatic carcinoma showing involvement of axillary lymphnode in 50% cases (9/18) and cervical lymphnodes (6/18) in 33.3% cases.

TABLE - IV : Distribution of all cases based on histological diagnosis.

Lesion	Histopathological profile of lymphadenopathy by paraffin section technique	
	No.of cases	Percentage
1. Inflammatory	75	65.8
- Chronic lymphadenitis	13	11.4
- Tuberculous lymphadenitis	53	46.5
- Reactive hyperplasia	9	7.9
2. Primary neoplasm	19	16.7
- Hodgkin's lymphoma (Mixed cellularity)	6	5.3
- Non Hodgkin's lymphoma	13	11.4
- Lymphocytic lymphoma (Well differentiated)	5	4.4
- Histiocytic lymphocytic lymphoma (Mixed cell type)	3	2.6
- Lymphoreticular lymphoma	4	3.5
- Lymphoblastic lymphoma (Poorly differentiated)	1	0.9
3. Secondary neoplasm	18	15.8
- Squamous cell carcinoma	6	5.3
- Adenocarcinoma	7	6.1
- Mucoid adenocarcinoma	4	3.5
- Myeloid leukaemic cell infiltration	1	0.9
4. Miscellaneous	2	1.7
- Angio follicular lymphnode hyperplasia	1	1.7
- Benign sinus histiocytosis	1	
Total	114	100.0

In this study evaluation of lymphnodes diagnosis was done by imprint cytology and their results were compared with frozen section and paraffin section, but final diagnosis was made by paraffin section, so we had made criteria of classification of various conditions diagnosed by histology using paraffin section.

As per our final diagnosis (Table-IV)by paraffin section technique, 75 (65.8%) cases of lymphadenopathy were inflammatory, 19 (16.7%) cases of primary neoplasm, 18 (15.8%) cases of secondary neoplasm (Metastatic) and 2 (1.7%) cases were placed in miscellaneous group.

Out of 75 cases of inflammatory lesions 13 cases (11.4%) were showing chronic lymphadenitis, 53 cases (46.5%) tuberculous lymphadenitis and 9 cases (7.9%) reactive hyperplasia. Out of 13 cases of chronic lymphadenitis one was of filarial lymphadenitis.

In the present study Rappaport's classification (1956) for Non Hodgkin's lymphoma and Rye modification of Lukes et al classification (1965) for Hodgkin's lymphoma was followed.

Among 19 cases of lymphoma 6 (5.3%) were of Hodgkin's lymphoma (all mixed cellularity type) and 13 (11.4%) were of non Hodgkin's lymphoma. In the Non Hodgkin's group lymphocytic lymphoma (well differentiated) were 5 (4.4%), histiocytic-lymphocytic (mixed cell type) were 3 (2.6%), lymphoreticular lymphoma were 4 (3.5%) and lymphoblastic lymphoma (poorly differentiated) was seen in 1 (0.9%) cases.

Out of 114 cases, 18 were of secondary neoplasia. In the secondary neoplasm groups metastatic squamous cell carcinoma were 6 (5.3%), adenocarcinoma were 7 (6.1%),mucoid adenocarcinoma were 4 (3.5%) (Confirmed by periodic acid shiff stain) and myeloid leukaemic cell infilatration was seen in 1 (0.9%) case.

Among miscellaneous group one case was of angio-follicular lymphnode hyperplasia and other case of benign sinus histiocytosis was found.

Imprint Cytology

In all cases imprint cytodiagnosis was done and results were confirmed by paraffin section technique. By imprint cytodiagnosis as shown in (Table-V), inflammatory lesions of lymphnodes were 75 (65.8%) which had included 18 (15.8%) case of chronic lymphadenitis and 57 (50%) case of tuberculous lymphadenitis.

21 (18.4%) cases were observed as a primary neoplasm in which 6 (5.3%) were of Hodgkin's lymphoma (all mixed cellularity) and 15 (13.1%) were on Non Hodgkin's lymphoma. Out of 15 cases of Hodgkin's lymphoma 7 (6.1%) were of lymphocytic lymphoma (well differentiated), 3 (2.6%) were of histiocytic lymphocytic (mixed cell type) lymphoma. 4 (3.5%) were of lymphoreticular lymphoma and 1 (0.9%) was of lymphoblastic lymphoma (poorly differentiated).

TABLE - V : Comparative study of lymphadenopathy by Imprint cytodiagnosis, Frozen section diagnosis and paraffin section diagnosis.

Lesions	Imprint Cytodiagnosis		Frozen section diagnosis		Paraffin section diagnosis	
	No.of cases	Percentage.	No.of cases	Percentage	No.of cases	Percentage
1. Inflammatory	75	65.8	77	67.5	75	65.8
- Chronic lymphadenitis	18	15.8	14	12.2	13	11.4
- Tuberculous lymphadenitis	57	50.0	53	46.5	53	46.5
- Reactive hyperplasia	-	-	10	8.8	9	7.9
2. Primary Neoplasm	21	18.4	19	16.7	19	16.7
- Hodgkin's lymphoma (Mixed cellularity)	6	5.3	6	5.3	6	5.3
- Non-Hodgkin's lymphoma	15	13.1	13	11.4	13	11.4
- Lymphocytic lymphoma (well differentiated)	7	6.1	5	4.4	5	4.4
- Histiocytic - lymphocytic lymphoma (mixed cell type).	3	2.6	3	2.6	3	2.6
- Lymphoreticular lymphoma	4	3.5	4	3.5	4	3.5
- Lymphoblastic lymphoma (Poorly differentiated)	1	0.9	1	0.9	1	0.9
3. Secondary Neoplasm	18	15.8	18	15.8	18	15.8
- Squamous cells carcinoma	6	5.3	6	5.3	6	5.3
- Adenocarcinoma	7	6.1	7	6.1	7	6.1
- Mucoid adenocarcinoma	4	3.5	4	3.5	4	3.5
- Myeloid leukaemic cell infiltration	1	0.9	1	0.9	1	0.9
4. Miscellaneous	-	-	-	-	2*	1.7

* - Angiofollicular lymphnode hyperplasia - 1 case.
- Benign sinus histiocytosis - 1 case.

Out of 18 cases of secondary neoplasm 6 (5.3%) were of metastatic squamous cell carcinoma, 7 (6.1%) were of adenocarcinoma, 4 (3.5%) were of mucoid adenocarcinoma and 1 (0.9%) was of myeloid leukaemic cell infiltration of the lymphnodes. All the cases of secondary neoplasm (metastatic) were correctly diagnosed which were confirmed by paraffin section.

TABLE - VI : Imprint cytodiagnosis and its accuracy

Imprint cytodiagnosis	Total No.of cases	Correct diagnosis by paraffin section	Misdiagnosed cases	Accuracy rate in percentage
Chronic lymphadenitis	18	10	8	55.6
Tuberculous lymphadenitis	57	50	7	87.7
Hodgkin's lymphoma (Mixed cellularity)	6	6	-	100.0
Non-Hodgkin's lymphoma	15	13	2	86.7
- Lymphocytic lymphoma (well differentiated)	7	5	2	
- Histiocytic-lymphocytic lymphoma (Mixed cell type)	3	3	-	
- Lymphoreticular lymphoma	4	4	-	
- Lymphoblastic lymphoma (Poorly differentiated)	1	1	-	
Metastatic	18	18	-	100.0
Total	114	97	17 (14.9)	Over all accuracy 85.1%

Table VI) shows imprint cytodiagnosis and its accuracy rate. In this study out of 18 cases of chronic lymphoadenitis when compared with paraffin section diagnosis 10 cases were correctly diagnosed and 8 were misdiagnosed, thus the accuracy rate in cases of chronic lymphadenitis was 55.6%. These 8 misdiagnosed cases include (Table VII) 4 of reactive hyperplasia, 3 of tuberculous lymphadenitis, and 1 of angiofollicular lymphnode hyperplasia.

Whereas in cases of tuberculous lymphadenitis, 50 were confirmed as tuberculous lymphadenitis by paraffin section method and 7 were misdiagnosed. The accuracy rate in cases of tuberculous lymphadenitis was 87.7%. Seven misdiagnosed cases were diagnosed by paraffin section technique as chronic lymphadenitis (3), reactive hyperplasia (3) & Benign sinus histiocytosis (1).

TABLE - VII : Cases misdiagnosed on imprint study.

Imprint diagnosis	Number of misdiagnosed cases	Paraffin diagnosis	
1. Chronic lymphadenitis	(8)	- Tuberculous lymphadenitis	(3)
		- Reactive hyperplasia	(4)
		- Angiofollicular lymphnode hyperplasia.	(1)
2. Tuberculous	(7)	- Chronic lymphadenitis	(3)
		- Reactive hyperplasia	(3)
		- Benign sinus histiocytosis	(1)
3. Non-Hodgkin's lymphoma	(2)	- Reactive hyperplasia	(2)

Figures in Parenthesis indicate number of cases.

Fifteen cases were observed in Non-Hodgkin's lymphoma by imprint cytodiagnosis, while 13 were confirmed as non Hodgkin's lymphoma, only 2 cases of lymphocytic lymphoma (Well differentiated) were confirmed as a reactive hyperplasia by paraffin section technique. Thus accuracy rate in cases of non Hodgkin's lymphoma by imprint cytology diagnosis was 86.7%.

The accuracy rate by imprint cytodiagnosis was 100% in case of Hodgkin's lymphoma and metastatic carcinoma.

RESULTS OF IMPRINT CYTOLOGY

Chronic lymphadenitis

In almost all cases the smears were hyper cellular, relatively increased number of large and small mature lymphocytes were seen along with other cells like neutrophils, eosinophils, histiocytes, plasma cells and reticulam cells. The large lymphocytes were differentiated from small lymphocytes by the presence of a definite rim of cytoplasm surrounding the nucleus (Microphotograph No.1).

Tuberculous lymphadenitis

In tuberculous lymphadenitis smears were markedly hypocellular with the presence of pink granular necrotic material, suggestive of caseous necrosis. However the most

significant and characteristic feature was presence of epitheloid cells, which were present in clusters or lying singly. These epitheloid cells contained voculated pink cytoplasm and big well defined nucleus having fine and stippled neuclear chromatin with one nucleolous in every nucleus. At places epitheloid cells were arranged in tubercle like fashion with Langhan's type of giant cells formation, beside this plasma cells, monocytes, neutrophils and eosinophils were also seen. Acid fast bacilli could not be demonstrated by special staining in any of the smears (Microphotograph No. 2,3).

Hodgkin's Lymphoma

All the 6 cases of Hodgkin's lymphoma were diagnosed by imprint cytodiagnosis which were confirmed by paraffin section. Four cases were male and 2 were females, while 3 were children and 3 belonging to middle age and in all cases generalised lymphadenopathy was seen. Out of 6 cases of Hodgkin's lymphoma, 5 had hepatosplenomegaly, duration of illness was ranging from 8 to 12 month. None of the patients having a picture of leukaemia and no abnormal cells were found in the peripheral blood, while all the patients were anaemic.

The smears in Hodgkin's lymphoma cases were characteristic by extreem hypercellularity with pleomorphic pattern with increased number of lymphocyts, reticulam cells,

plasma cells, and eosinophils. The reticulam cells showed greater variability in shape and size, but the most diagnostic feature was the presence of Reed sternberg type of giant cell which appeared quite large with extensive pale cytoplasm and mirror image type of nuclei containing prominent nucleoli. These cases were diagnosed as Hodgkin's lymphoma of mixed cellularity type (Microphotograph No. 4).

NON-HODGKIN'S LYMPHOMA

Lymphocytic lymphoma (well differentiated):

Seven cases of lymphocytic lymphoma (well differentiated) were diagnosed by imprints. The age of 7 patients of lymphocytic lymphoma varied from 45 to 58 years, and all were males, in which cervical axillary, or generalised lymphnode enlargement was found. Duration of illness was short (6-8 month), none of the patient presented a picture of leukaemia and no abnormal type of cells seen in peripheral blood. In two cases, hepatosplenomegaly had seen. The smears were shwoing extreme hypercellularity which also tended to look monomorphic in character showing little pleomorphism. Characteristic cell were immature and mature small lymphocytes uniformly scattered and having scanty blue cytoplasm and round dark hyperchromatic nuclei and the nuclei showed a regular and round contour with one or two nucleoli and condensed chromatin network (Microphotograph No. 5).

Histiocytic-lymphocytic lymphoma (Mixed cell type) :

The age of 3 patients of histiocytic lymphoma varied from 25 to 40 years, all were females in which cervical and axillary lymphnode enlargement was seen. Duration of illness was short 3-5 month. None of the patients presented a picture of leukaemia and no abnormal cells were seen in peripheral blood.

The smears were hypercellular with pleomorphic picture showing a diffuse pattern of neoplastic large cells, histiocytes with abundant greyish blue cytoplasm and with indented nuclei, in which 2-4 neucleoli were present. Along with it large number of lymphocytes were also found Reticulin (Microphotograph No. 6).

Lymphoreticular lymphoma :

In all patients of lymphoreticular lymphoma age was varying from 8 to 58 years 2 were adult males aged (47-58 years respectively), one was child age (8 years) and one female aged 55 years presenting with generalised and cervical lymphadenopathy. In one female case there was intra abdominal mass of 5 month duration with huge hepato-splenomegaly. All the patients were moderately anaemic, but no abnormal cells could be made out in the peripheral blood smear.

Smears from these cases were markedly hypercellular and pleomorphic as compared to lymphocytic lymphoma, fair number of mitotic figure were also seen. Most of the cells were reticulam cells, and lymphoblast. The reticulam cells were having markedly irregular, hyperchromatic nuclei with numerous coarse chromatin clumps and occasional prominent nucleoli. The lymphoblast were larger in size than the large lymphocytes and had a big round dark hyperchromatic irregular nucleous and nucleoli with a coarse chromatin. Cytoplasm was santy, deep blue.(Microphotograph No. 7).

Lymphoblastic lymphoma (Poorly differentiated) :

Only one case aged 60 years male with mesentric lymphnode enlargement, diagnosed as poorly differentiated lymphoma, duration of illness was short (5 months). On peripheral blood examination, there was no abnormal type of cells seen. The smear of this case was characterised by extreme hypercellularity which also tend to took monomorphic in character, there was predominance of cells of lymphocytic series in which more than 80% were lymphoblasts. The lymphoblasts were little larger than large lymphocytes and had a big round or irregular nucleus nucleoli with a stippled coarse chromatin and 1 to 3 distinct nucleoli the cytoplasm was scanty deep blue and in some cells it was granular (Microphotograph No. 8).

Lymphnodes with metastatic lesion :

All 18 cases of this group were confirmed by paraffin section. The diagnosis from the impression smears were quite apparent in all these cases. The diagnosis of epidermoid or squamous cell carcinoma was made by the presence of alien tumour cells having large pleomorphic cells and a big hyperchromatic nuclei. The nuclear borders were irregular. The cytoplasm was pink in colour suggestive of keratinization (Microphotograph No. 9).

In case of metastatic adenocarcinoma, the tumour cells were pleomorphic, round to oval in shape. They had prominent hyperchromatic basal nuclei which had 1 or 2 distinct nucleoli. The cytoplasm was pale pink and abundant in some cells. They were singly distributed tended to form acini. In some instances the number of alien malignant epi. thelial cells were more than the native population of the lymphocytes while in others they were present in sufficient numbers to form a definite diagnosis (Microphotograph No. 10).

In mucoid adenocarcinoma cells contained ecentric nuclei and the cytoplasm was vocuolated filled with mucin. These cells were P.A.S. positive.

The leukaemic cells infiltration of lymph node smear was showing fair number of myeloid series of cells including myeloblast, myelocyte and melomyelocyte, and on the findings of peripheral blood, diagnosis of chronic myeloid leukaemia was made.

TABLE - VIII : Frozen section diagnosis and its accuracy

Frozen section diagnosis	Total No.of cases	Correct diagnosis by paraffin section	Misdiagnosed cases	Accuracy rate in percentage
Chronic lymphadenitis	14	13	1	92.8
Tuberculous lymphadenitis	53	53	-	100.0
Reactive hyperplasia	10	9	1	90.0
Hodgkin's lymphoma (Mixed cellular type)	6	6	-	100.0
Non-Hodgkin's lymphoma :	13	13	-	
- Lymphocytic lymphoma (Well differentiated)	5	5	-	
- Histiocytic lymphocytic lymphoma (Mixed cell type)	3	3	-	
- Lymphoreticular lymphoma	4	4	-	100.0
- Lymphoglastic lymphoma (Poorly differentiated)	1	1	-	
Metastatic lesion	18	18	-	
- Squamous cell carcinoma	6	6	-	
- Adenocarcinoma	7	7	-	
- Mucoid adenocarcinoma	4	4	-	100.0
- Myeloid leukaemic cell infiltration	1	1	-	
Total	114	112	2	98.2 (Overall accuracy)

TABLE- IX : Cases misdiagnosed on frozen section

Frozen section diagnosis	Misdiagnosed case	Paraffin section diagnosis
Chronic lymphadenitis	1	Angiofollicular lymphnode hyperplasia
Reactive hyperplasia	1	Benign sinus histiocytosis

By frozen section diagnosis as shown in Table-V inflammatory lesion of lymphnodes were 77 (67.5%) which had included 14 cases (12.2%) of chronic lymphadenitis, 53 cases (46.5%) of tuberculous lymphadenitis and 10 (8.8%) cases of reactive hyperplasia.

19 (16.7%) cases were observed as a primary neoplasm in which 6 (5.3%) were of Hodgkin's lymphoma (all mixed cellularity) and 15 (13.1%) were of non-Hodgkin's lymphoma including 5 (4.4%) of lymphocytic lymphoma (well differentiated). 3(2.6%) of histiocytic- lymphocytic (mixed cell type) lymphoma, 4 (3.5%) of lymphoreticular lymphoma and 1 (0.9%) was of lymphoblastic lymphoma (Poorly differentiated).

Out of 18 cases of secondary neoplasm, 6 (5.3%) were of metastatic squamous cell carcinoma, 7 (6.1%) were of adenocarcinoma, 4 (3.5%) were of mucoid adenocarcinoma and 1 (0.9%) was of myeloid leukaemic cell infiltration of the lymphnode. All the cases of secondary neoplasm (Metastatic) were correctly diagnosed which were confirmed by paraffin section.

(Table - VIII & IX) shows frozen section diagnosis and its accuracy rate, chronic lymphadenitis was obserbed in 14 cases in which correct diagnosis were made by paraffin section in 13 cases only, one misdiagnosed case was of angio-follicular lymphnode hyperplasia. Thus the accuracy rate of chronic lymphadenitis was 92.8%.

The frozen section of chronic lymphadenitis showed hyperplasia of the reticulo-endothelial cells; large number of endothelial cells become swollen, rounded and cast off into greatly dilated lymph sinus, and sinus were crowded with polymorphonuclear leucocytes, histiocytes and eosinophils. In one case of chronic lymphadenitis adult filarial worm was seen which was confirmed by paraffin section. (Microphotograph No - 11).

Tuberculous lymphadenitis was observed in 53 (46.5%) cases, and all cases were confirmed by paraffin section. Thus the accuracy rate of tuberculous lymphadenitis was 100%. The frozen section of tuberculous lymphadenitis showed typical tubercle. Caseous necrosis was surrounded by chronic inflammatory cells including epithelioid cells, lymphocytes and the presence of typical Langhan's giant cells confirm the diagnosis of tuberculous lymphadenitis. In few cases where massive caseous necrosis was observed, much of the structure of the lymph node had disappeared. (Microphotograph-12)

10 cases (8.8%) of reactive hyperplasia were diagnosed by frozen section technique, in which 9 were

confirmed by paraffin sections. One case was diagnosed as reactive hyperplasia by frozen section and later on by paraffin section found to be a case of benign sinus histiocytosis. Thus the accuracy rate in reactive hyperplasia was 90%. Frozen section in cases of reactive hyperplasia revealed lymphoid follicles with large number of germinal centre exhibiting a high mitotic activity and numerous macrophages and plasma cell. The lymphoid follicles vary considerably in size but have well defined margin surrounded by a mantle of small lymphocytes (Microphotograph No. 13, 14).

The frozen section of all the 6 cases of Hodgkin's lymphoma (Mixed cellularity) were confirmed by paraffin section. The accuracy of frozen section technique in case of Hodgkin's lymphoma was 100%. In the frozen section of Hodgkin's lymphoma (mixed cellularity) the picture was hypercellular and pleomorphic, architecture of the lymphnodes was obliterated by proliferating lymphocytes, histiocyte, eosinophils, polymorphonuclear leucocytes and plasma cells. The Hodgkin's lymphoma was confirmed by the presence of diagnostic Reed sternberg giant cells (Microphotograph No. 15).

All the 13 cases of Non-Hodgkin's lymphoma were correctly diagnosed by frozen section technique and were further labelled as lymphocytic lymphom (well differentiated) 4, histiocytic-lymphocytic lymphoma (mixed cell type) 3, lymphoreticular lymphoma 4, and lymphoblastic lymphoma (Poorly differentiated) one. The findings were confirmed by paraffin section and accuracy rate was 100% (~~Microphotograph No.~~ Microphotograph No. 16, 17, 18, 19).

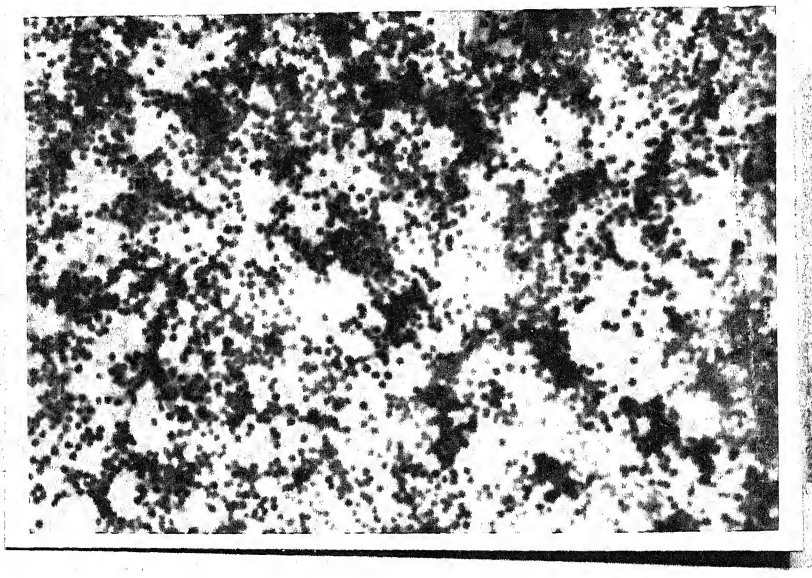
Out of 18 cases (15.8%) of metastatic carcinoma diagnosed by frozen section, 6 were of squamous cell carcinoma and 7 were of adenocarcinoma and 4 were of mucoid adenocarcinoma and in one case myeloid leukaemic cells infiltration was seen. All the cases were correctly diagnosed, which were later on confirmed by paraffin sections. Thus the accuracy rates was 100% in metastatic carcinoma.

The frozen section of metastatic squamous cell carcinoma showed replacement of normal pattern by squamous cells and there were large number of epithelial pearls. The squamous cells showed fair degree of anisocytosis and anisonucleosis. In between these group of squamous cells few lymphoid follicle were seen; some of which had active germinal centre and few of them atrophied. In the lymphatic sinuses there were collection of inflammatory cells including lymphocytes and plasma cells (Microphotograph No. 20).

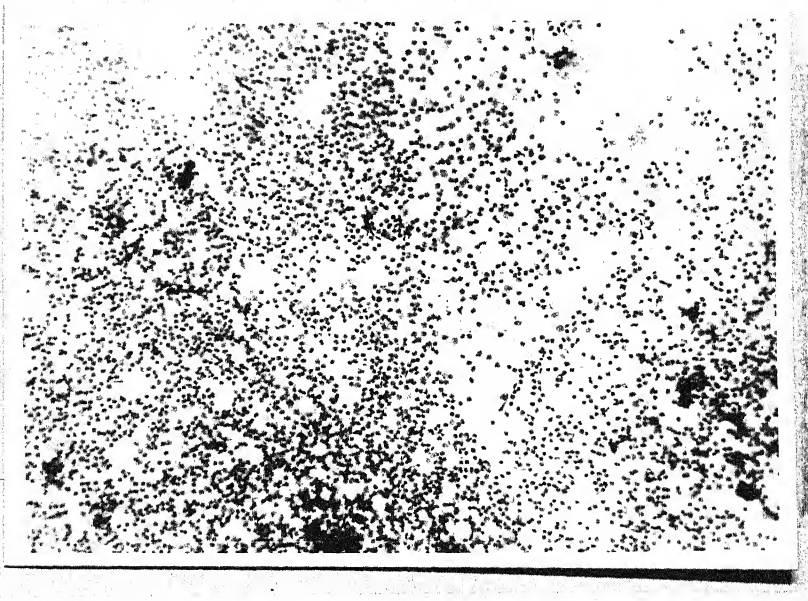
In the metastatic adenocarcinoma the normal pattern of lymph node was greatly destroyed and replaced by groups of malignant cells. At places these cells were arranged in the form of acini of variable shape and size. In case of mucoid adenocarcinoma the pattern was similar to that of adenocarcinoma as described above, but the cells were having ample cytoplasm with vacuolation. The cells were PAS positive in paraffin section. (Microphotograph NO 21).

In summerising the results of above study we can conclude that imprint cytodiagnosis giving 100% accuracy rate in cases of Hodgkin's lymphoma and secondary or metastatic carcinomas while in non-Hodgkin's lymphoma accuracy rate was 86.7%. In benign conditions like tuberculosis accuracy rate was 87.7% and chronic lymphadenitis 55.6% and in total study of 114 cases over all accuracy rate was 85.1%.

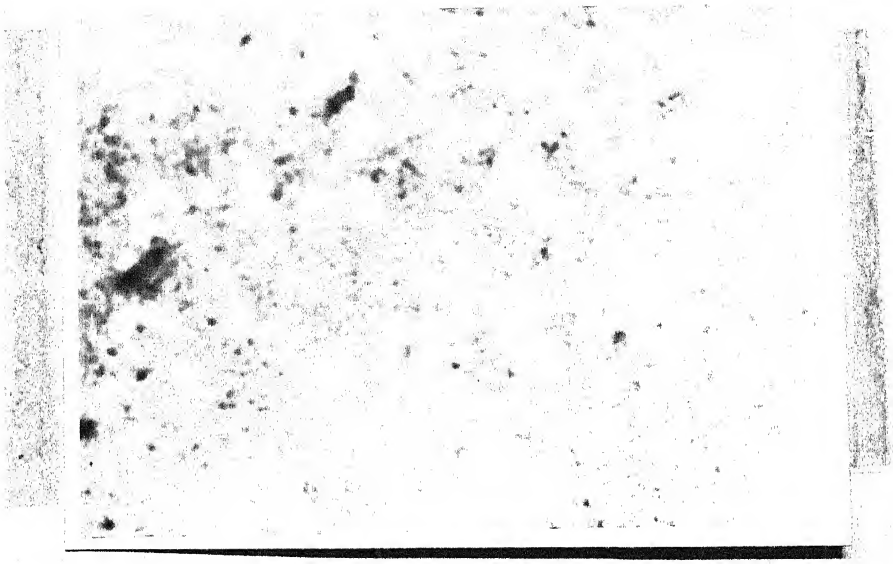
While in frozen section study accuracy rate was 100% in cases of Hodgkin's lymphoma, Non-Hodgkin's lymphoma, secondary metastatic carcinoma and tuberculous lymphadenopathy. In chronic lymphadenitis accuracy rate was 92.8% and in reactive hyperplasia 90% so in total cases over all accuracy rate was 98.2%.



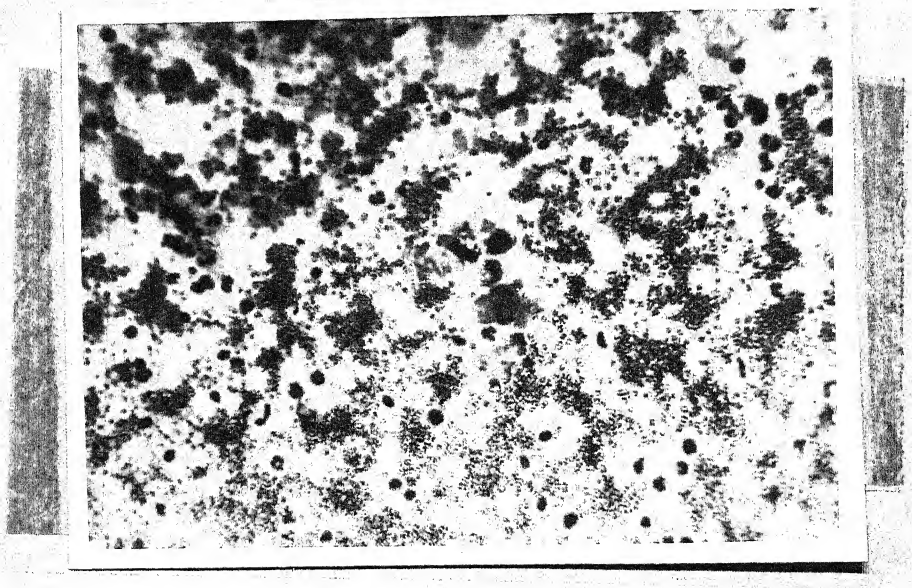
Microphotograph No.1: Imprint smear of chronic lymphadenitis with marked hypercellularity (H & E X 200).



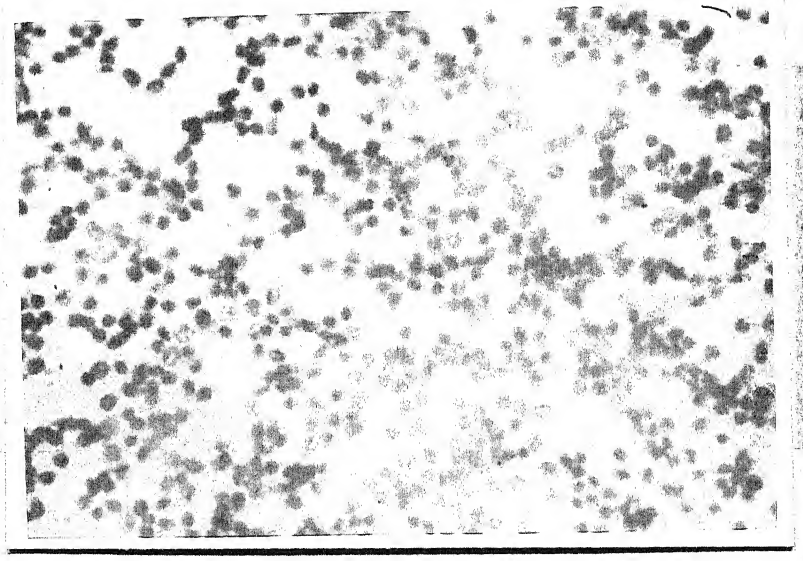
Microphotograph No.2: Imprint smear of tuberculous l lymphadenitis showing Langhan's giant cells, lymphocytes and epitheloid cell (H & E X 100).



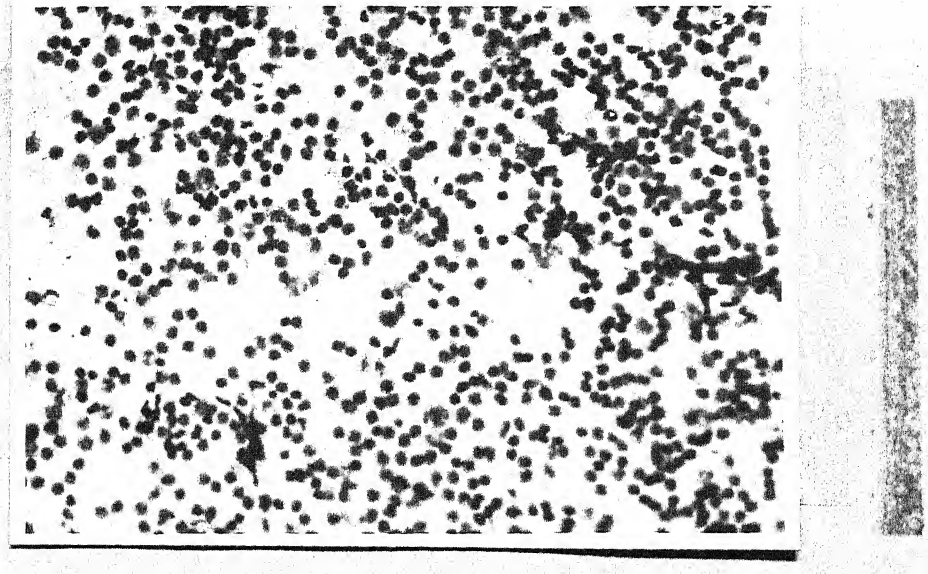
Microphotograph No.3: Imprint smear of tuberculous lymphadenitis showing Langhan's giant cells, lymphocytes and epitheloid cells (H & E X 200).



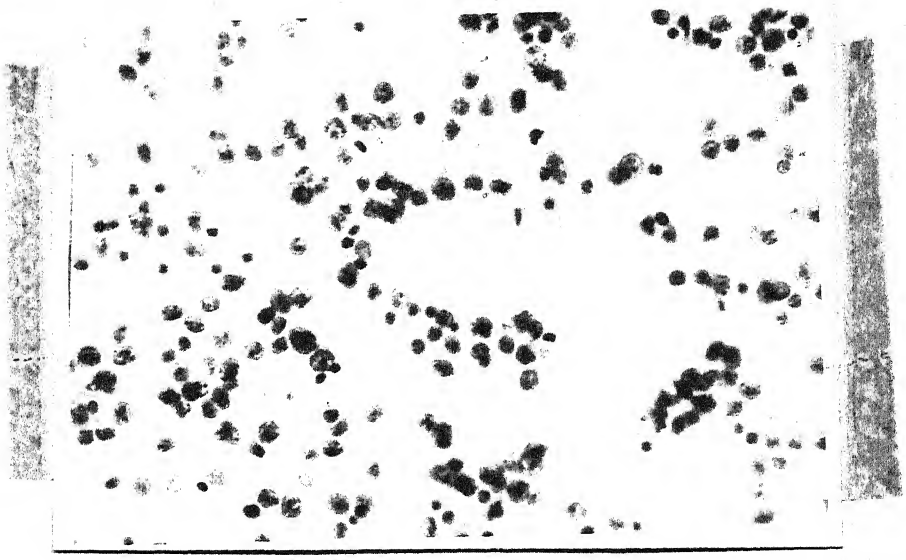
Microphotograph No.4: Imprint smear of Hodgkin's lymphoma showing pleomorphic picture with Reed-Sternberg giant cells. (Pap X 400)



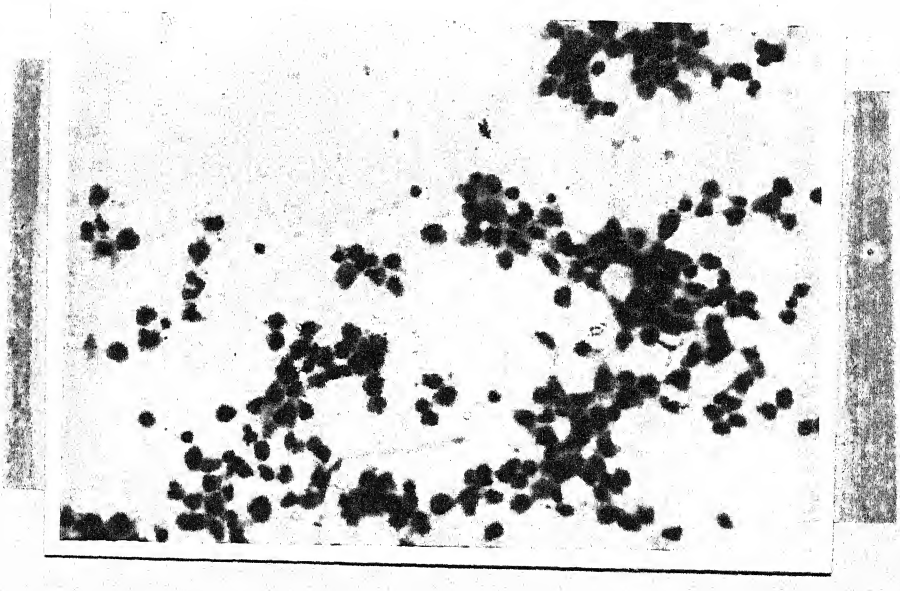
Microphotograph No.5: Imprint smear of lymphocytic lymphoma, showing monomorphic picture with large number of lymphocyte (H & E, X 400).



Microphotograph No.6: Imprint smear of mixed Non-Hodgkin's lymphoma showing malignant histiocytes and lymphocytes (Pap, X 250).

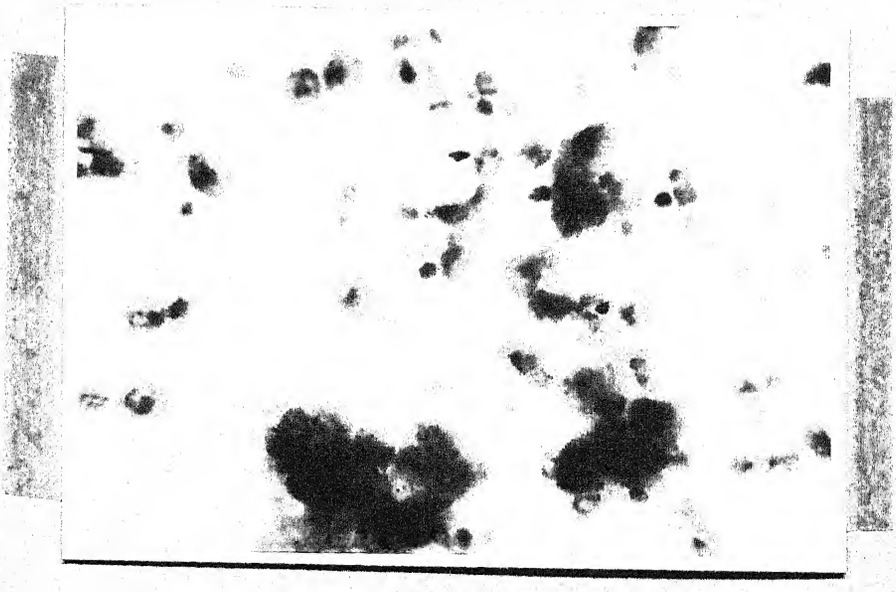


Microphotograph No.7:Imprint smear of lymphoreticular lymphoma showing pleomrophic appearance with mostly malignant reticulam cells and lymphocytes (H & E, X 400).

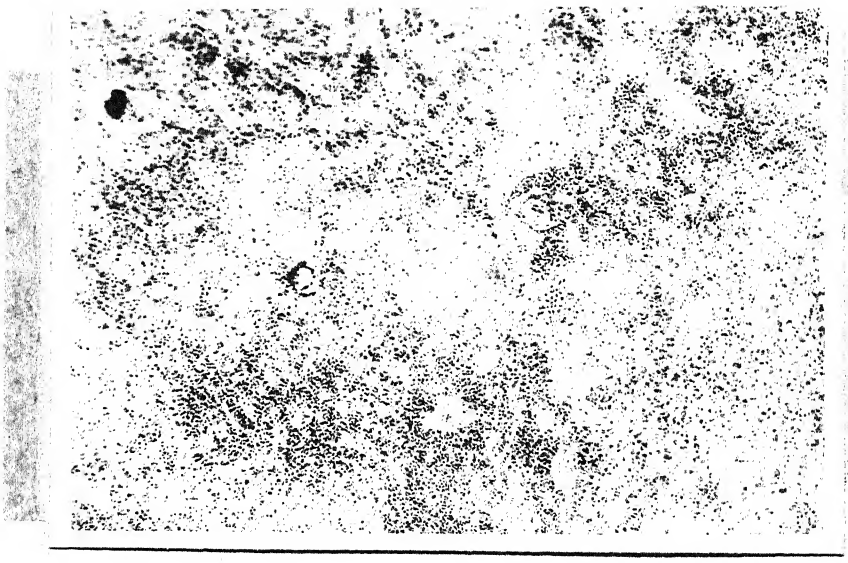


Microphotograph No.8:Imprint smear of lymphoblastic lymphoma showing predominent number of lymphoblast (Pap, X 400).

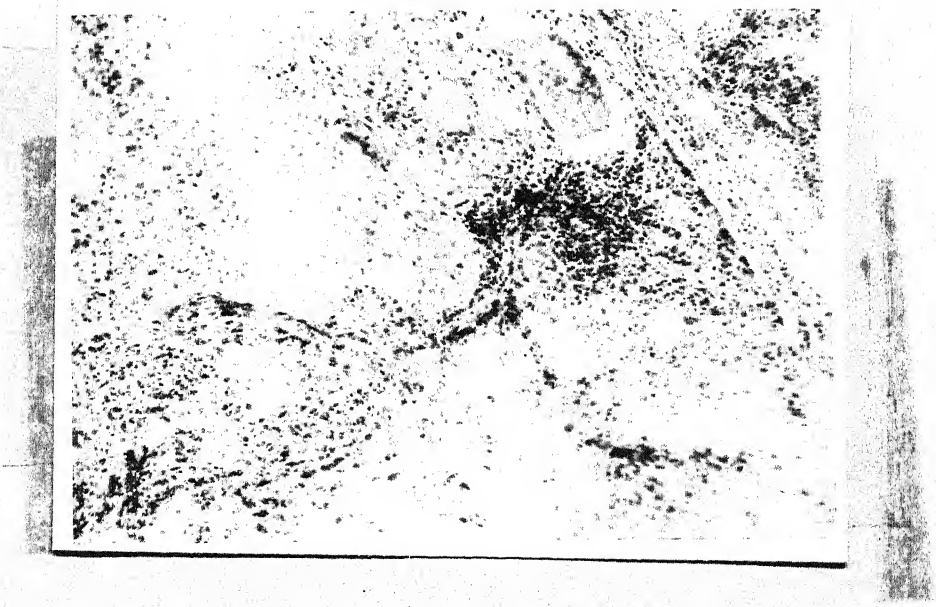
Microphotograph No.9:Imprint smear of metastatic squamous cell carcinoma. The malignant cells are solitary as well as in clumps (Pap X 320).



Microphotograph No.10:Imprint smear of metastatic adenocarcinoma showing oval cells with hyper-chromatic nuclei arranged in acinic pattern and solitary. (H & E, X 400).

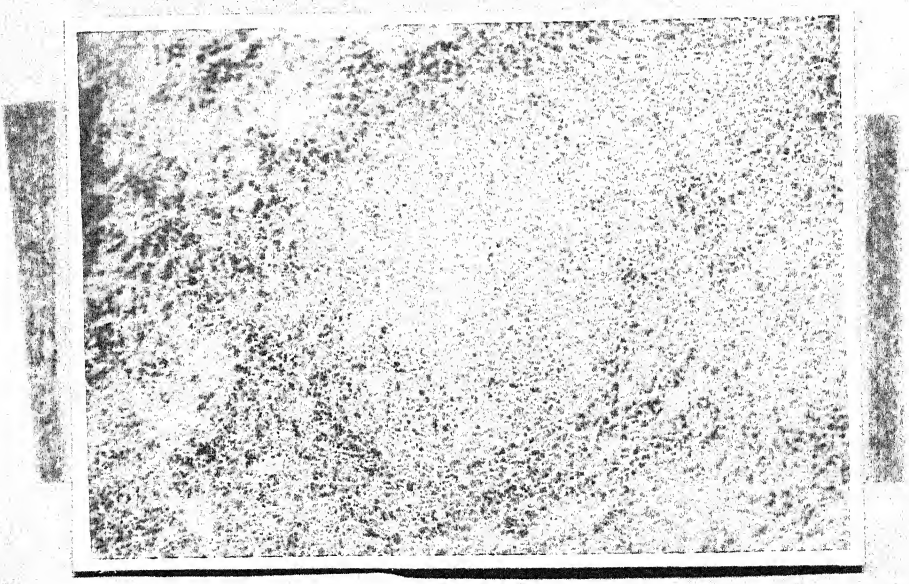


Microphotograph No.11: Frozen section of Chronic lymphadenitis (H & E, X 100).

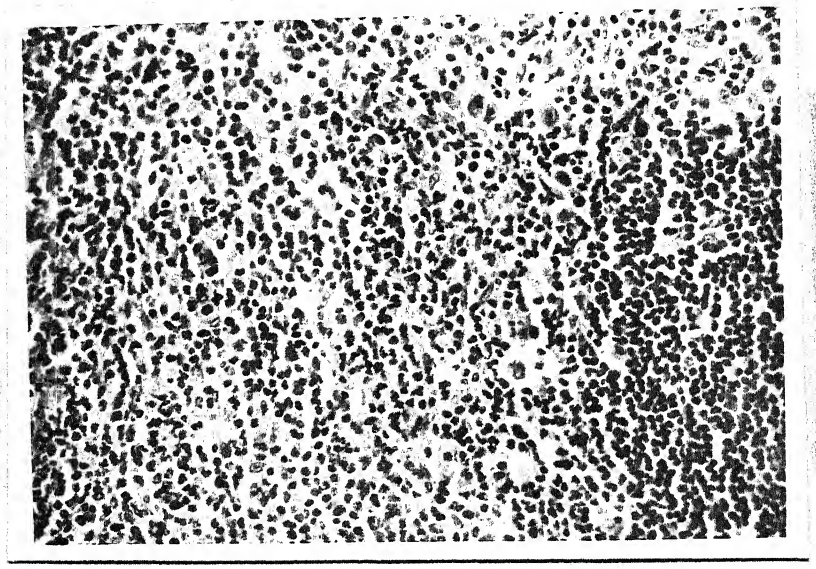


Microphotograph No.12: Frozen section showing tuberculous lymphadenitis showing tubercle along with giant cell (H & E, X 170).

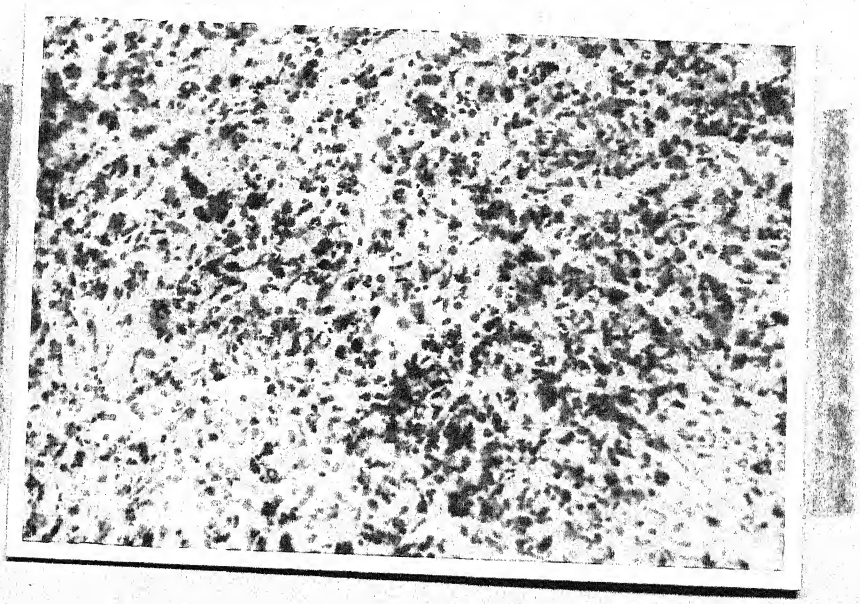
Microphotograph No.13: Frozen section showing reactive hyperplasia (H & E, X 68).



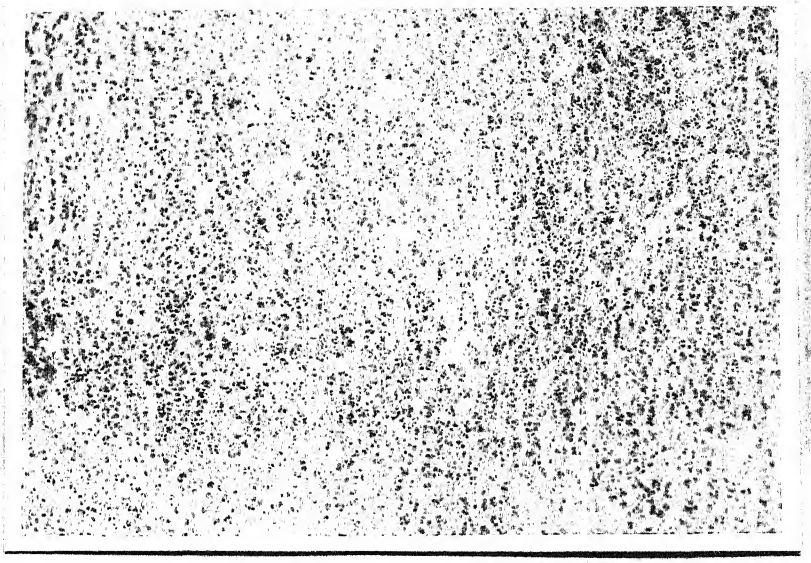
Microphotograph No.14: Frozen section showing reactive hyperplasia (H & E X 100)



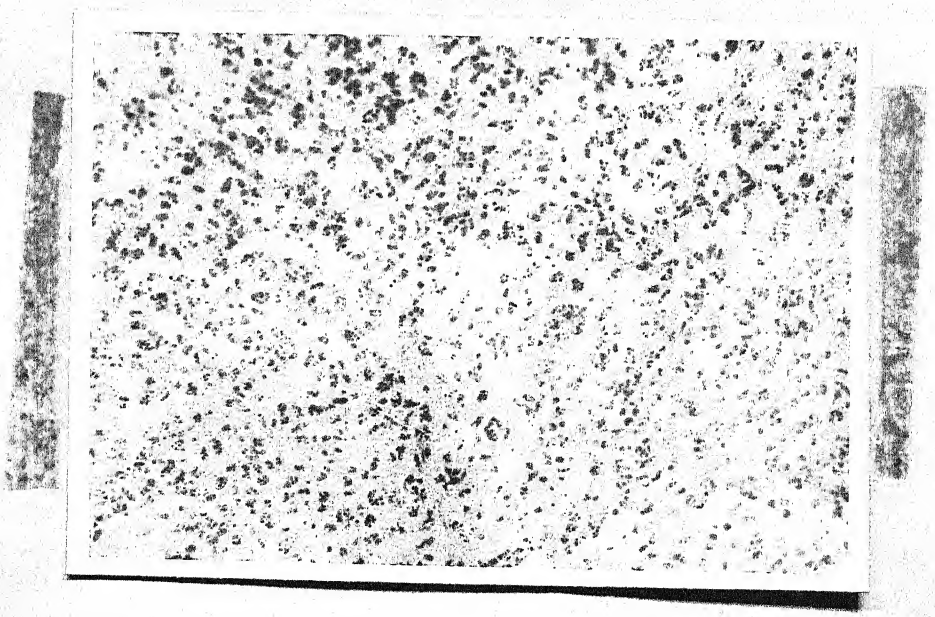
Microphotograph No.15: Frozen section of Hodgkin's lymphoma showing mixed cellular picture along with Reed-Sternberg giant cells (H & E X 320).



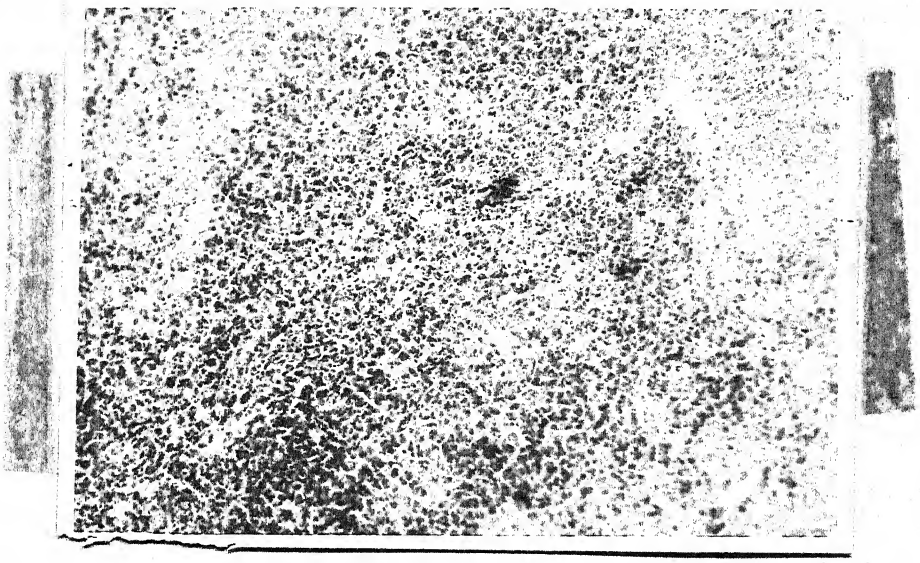
Microphotograph No.16: Frozen section showing non-Hodgkin's lymphoma mixed type (H & E, X 170).



Microphotograph No.17: Frozen section showing lymphoreticular lymphoma (H & E, X 100).

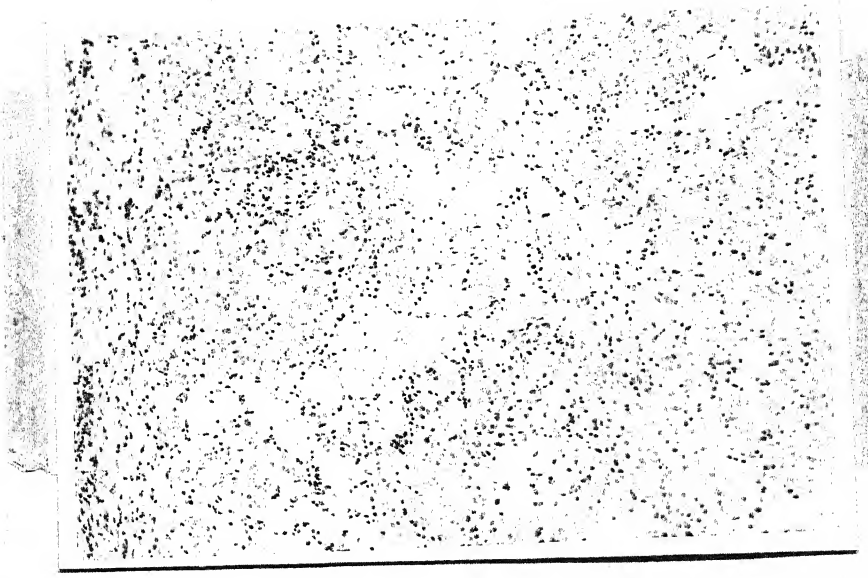


Microphotograph No.18: Frozen section showing lymphoreticular lymphoma (H & E, X 200).

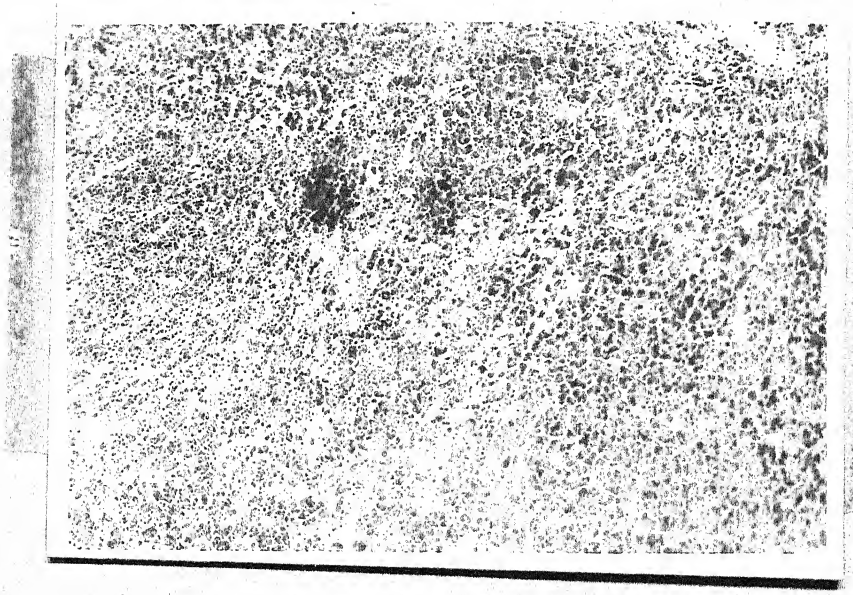


Microphotograph No.19: Frozen section showing non-Hodgkin's lymphoma, lymphoblastic type (H & E, X 170).

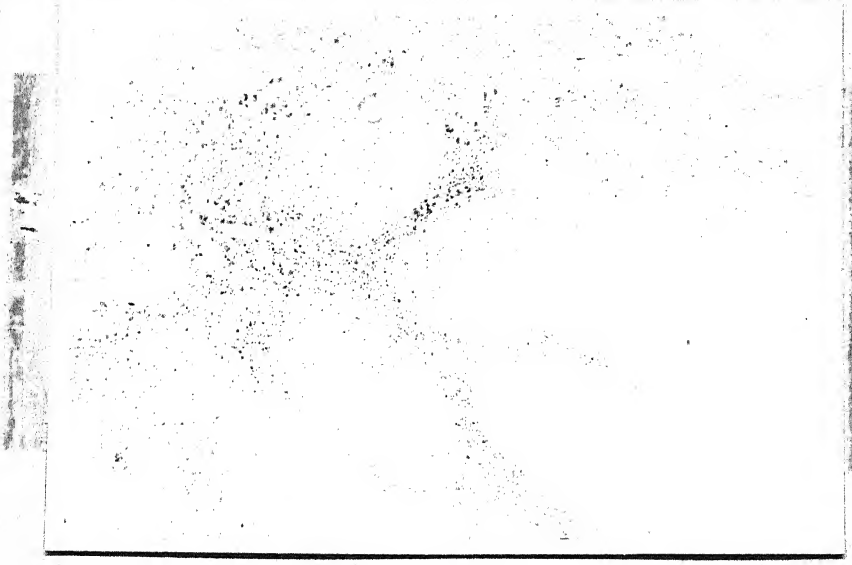
Microphotograph No.20: Frozen section showing metastatic squamous cell carcinoma (H & E, X 170).



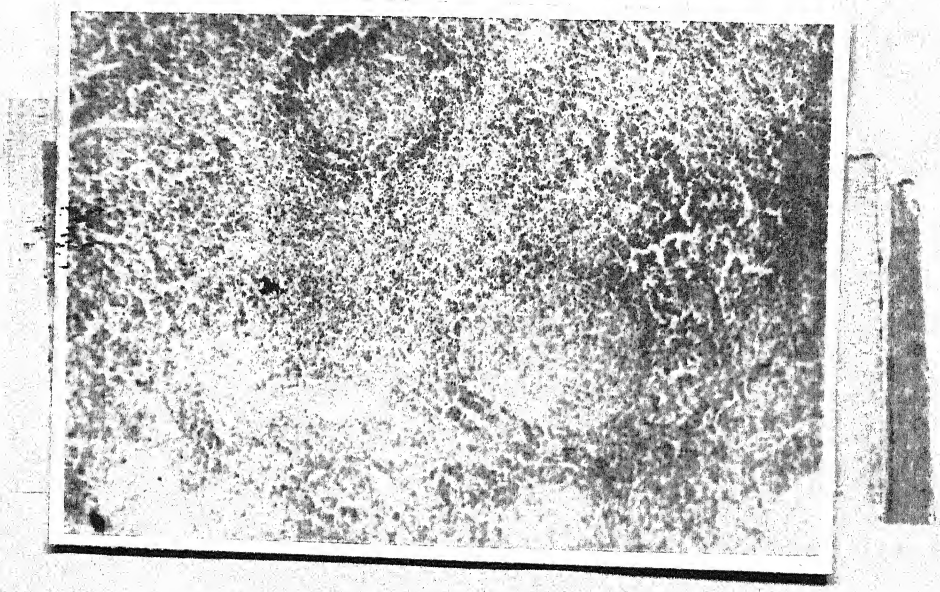
Microphotograph No.21: Frozen section showing metastatic adenocarcinoma (H & E, X 170).



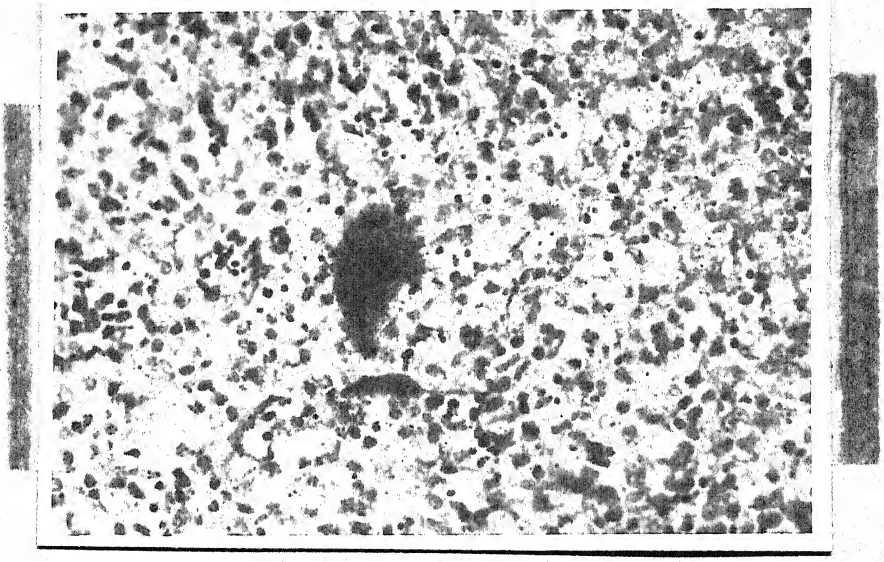
Microphotograph No.22: Paraffin section showing chronic lymphadenitis (H & E, X 100).



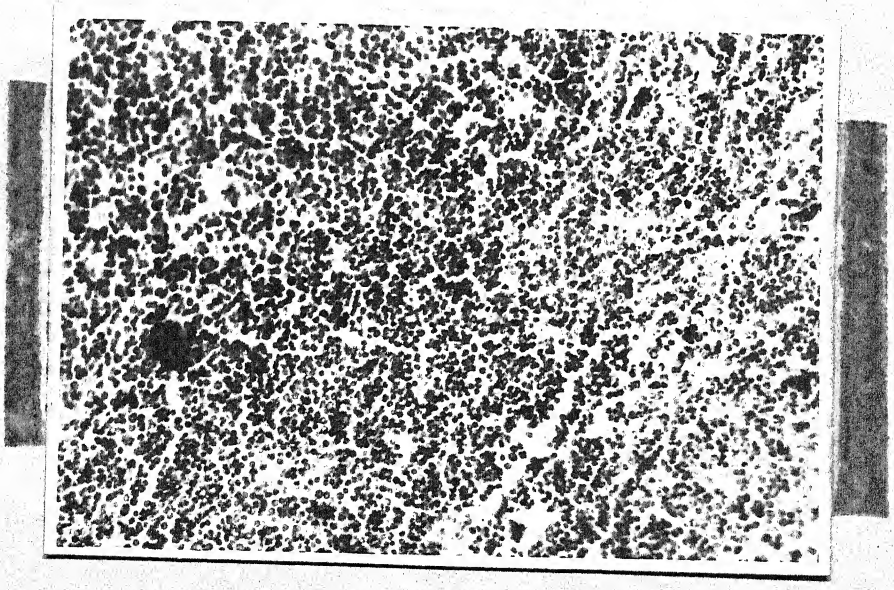
Microphotograph No.23: Paraffin section showing tuberculous lymphadenitis (H & E, X 100).



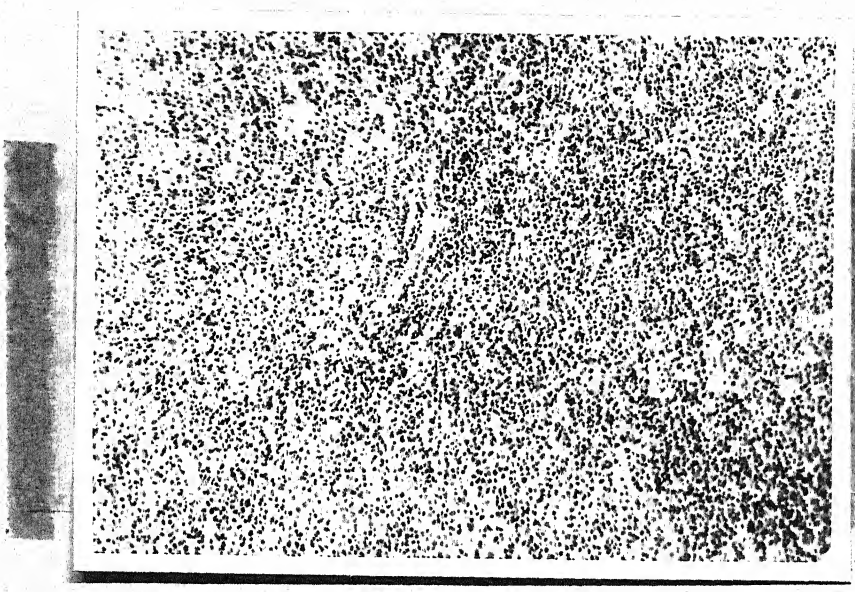
Microphotograph No.24: Paraffin section showing reactive hyperplasia (H & E, X 100).



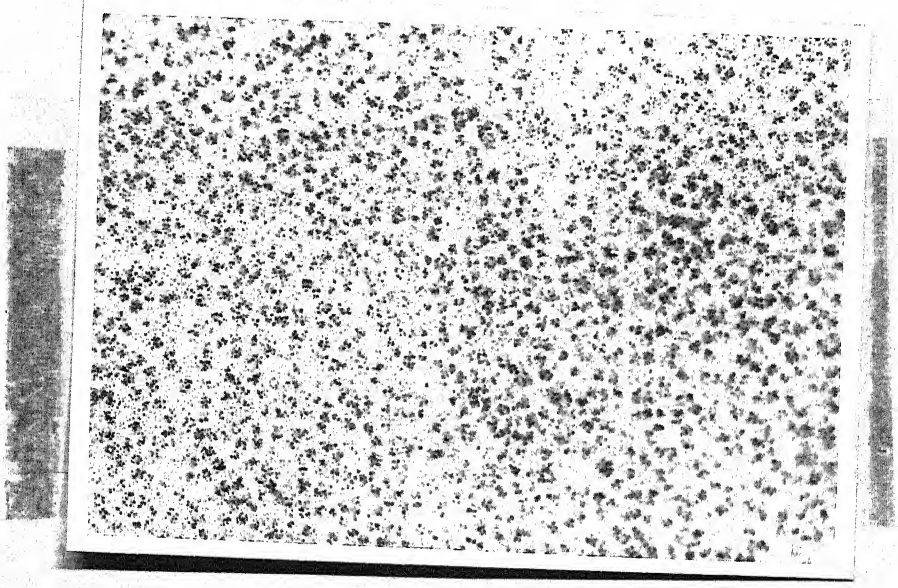
Microphotograph No.25: Paraffin section showing Hodgkin's lymphoma (mixed cellularity) (H & E, X 400).



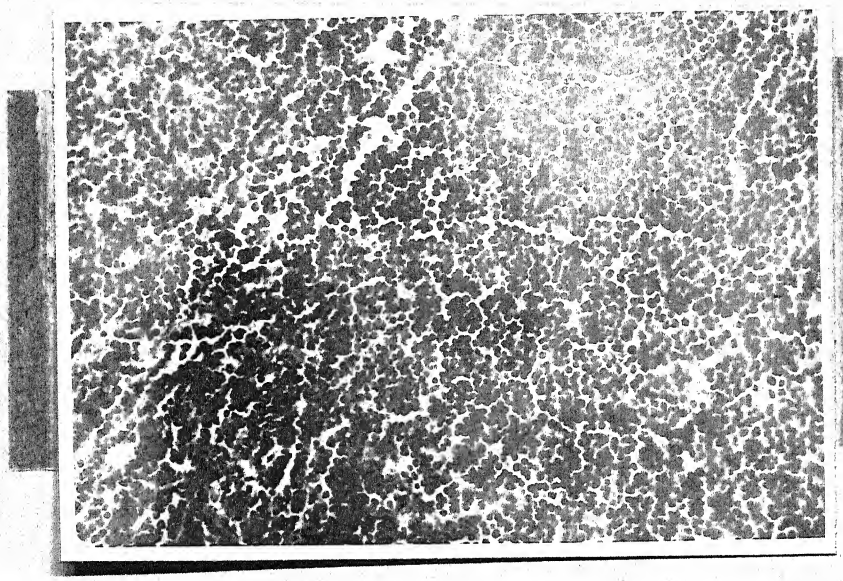
Microphotograph No.26: Paraffin section showing lymphocytic lymphoma well differentiated (H & E, X 170)



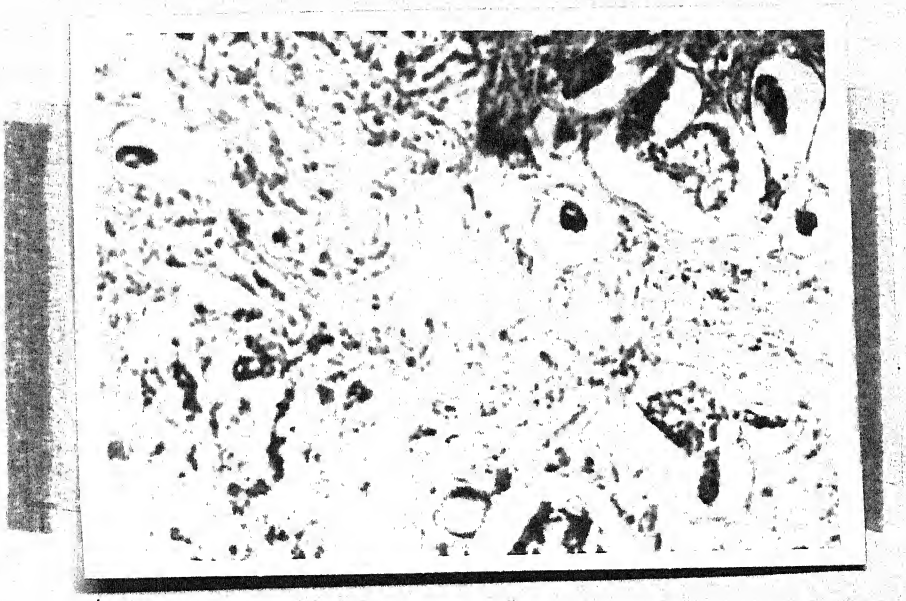
Microphotograph No. V: Paraffin section showing non-Hodgkin's lymphoma mixed type (H & E, X 100).



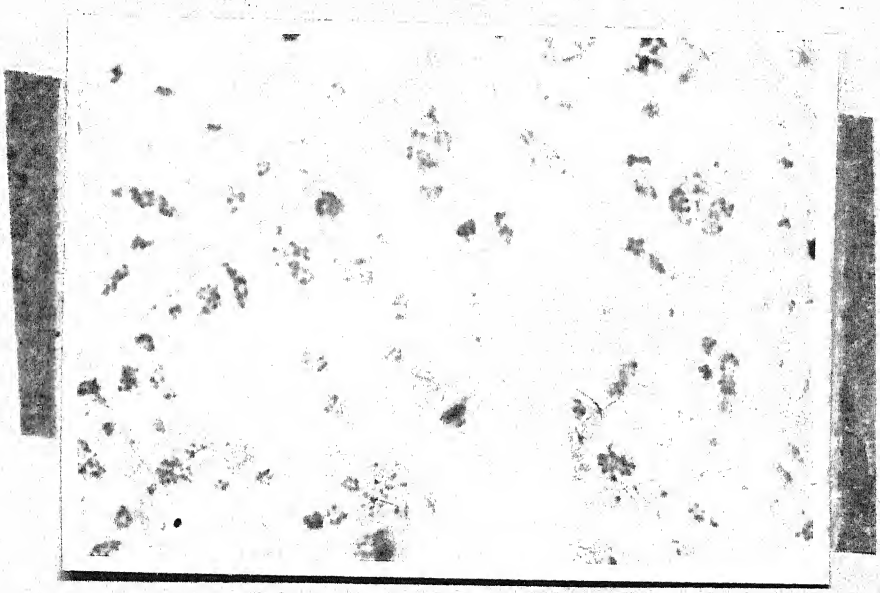
Microphotograph No. 28 Paraffin section showing lymphoreticular lymphoma (H & E, X 250).



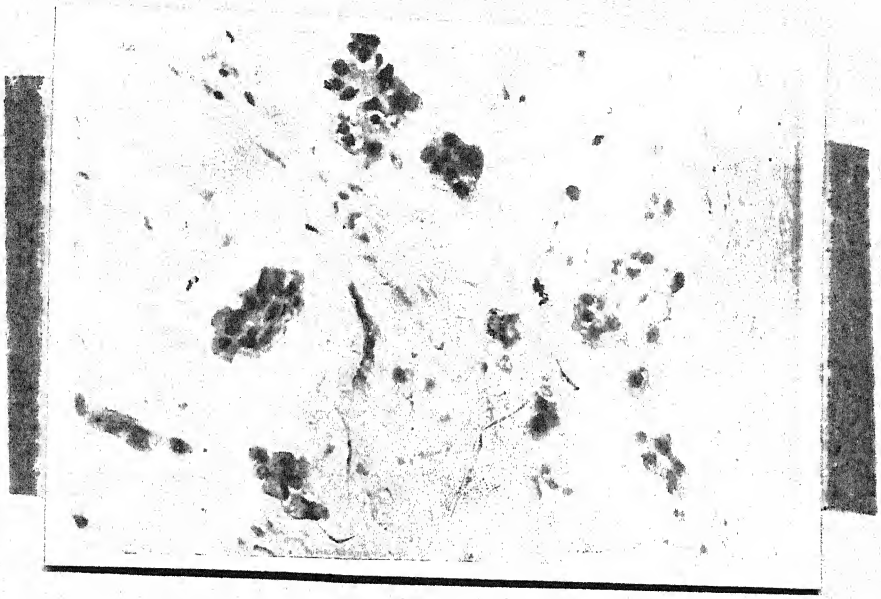
Microphotograph No.29:Paraffin section showing lymphoblastic lymphoma (H & E, X 170).



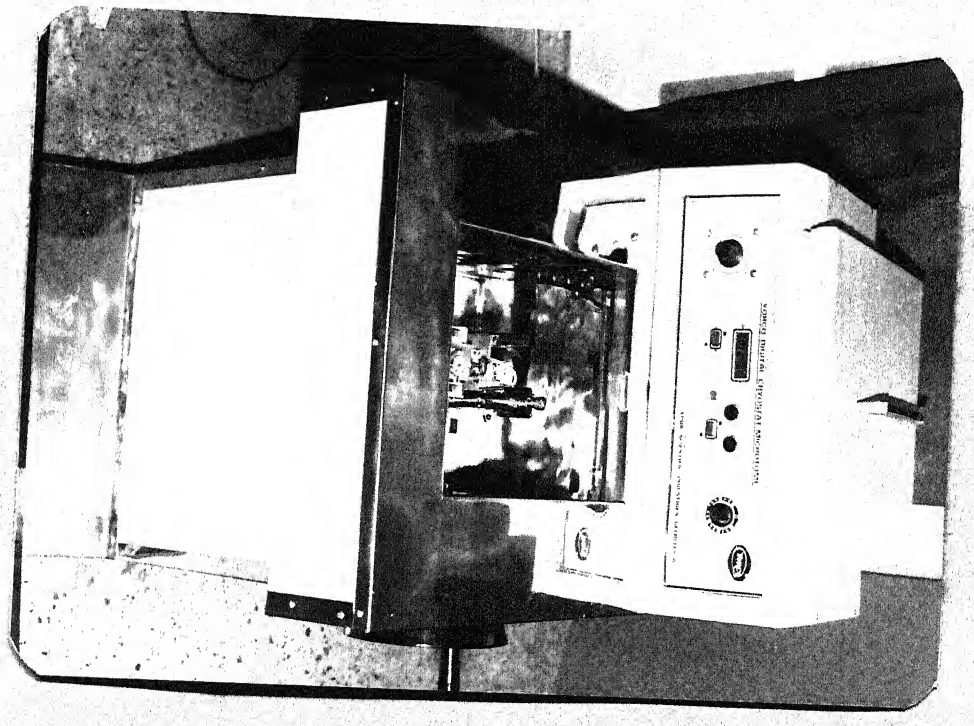
Microphotograph No.31: Paraffin section showing metastatic adenocarcinoma (H & E, X 170).



Microphotograph No.32 Paraffin section showing metastatic mucoid adenocarcinoma (H & E, X 170)



Microphotograph No.33:Paraffin section showing metastatic mucoid adenocarcinoma (H & E, X 250).



30: CRYOSTAT MICROTOME

D I S C U S S I O N

D I S C U S S I O N

An array of the diseases in human beings are manifested more or less as enlargement of the lymphnodes either primarily or secondarily, and that's why the study of lymphnodes is an exclusive need and the proper procedure for the clinical diagnosis of both medical as well as surgical point of view. It is the pathologist whose meticulous study by various procedures e.g. imprint cytology, frozen section and paraffin section technique, not only assures the clinician about the various aspedts of the disease and its diagnosis but also helps them in providing the guideline for the treatment.

Thus the pathologists responsibility is great and task difficult because of inherent problems in obtaining standard and excellent section from fresh tissue. An accuracy of such study may be improved and difficulties may be reduced by supplementing the tissue slides with imprint cytology and frozen sections.

In light of above facts, present study has attempted to achieve the exact diagnosis of the lymphnode submitted for examination. Three important procedures like; imprint cytology, frozen section and paraffin section technique have been undertaken and their comparative study has been done on the basis of the diagnosis worked out, taking the paraffin section technique as standard one for final diagnosis.

A total of 114 cases were included in the present study. Out of which a large number was constituted by those of cervical lymphnodes 39.5%, followed by axillary lymphnodes 28.0%, the minimus involvement 5.3% was seen in case of mesentric lymphnodes. Gupta et al (1988) also reported maximum cases of lymphadenopathy in cervical group followed by abdominal groups.

In the present study lymphadenopathy predominance in females as compared to males (65.5% female V/s 35.5% males) was seen. Maximum cases of lymphadenopathy were found in 11-30 years age groups and minimum cases of lymphadenopathy were found in 60-70 years group.

Histopathological examination of the lymphnodes by various technique's revealed that imprint cytology is reasonably simple and quick procedure for early diagnosis at the same time frozen section is also giving quick and early diagnosis.

In the study of imprint cytodiagnosis of lymph nodes multiple stains viz. Haematoxyline and eosin, Papanicolaou and Zeihl-Neelson staining (in case of tuberculous lymphadenitis) are used giving more information. Ultmann (1958), Agarwal et al (1977) were also used multiple staining viz. Haematoxyline and eosin, papanicolaou and Giemsa stain and reported more information than using only the

conventional haematoxyline and eosin stain. Haematoxyline and eosin stain in our opinion gabe better results in comparison to papanicolaou, because the nucleous and cytoplasm of the cells are more clearly visualised. Lucas (1955), Ultmann et al (1958), Agarwal et al (1977) found that Papanicolaou's stain gave better results in comparison to haematoxyline and eosin stain. Chaudhary (1984) also used multiple stain and found identical results.

On cytological examination of imprints, standard criteria were used to classify the cells as benign and malignant.

1. The malignant cells wer invariably seen in clumps while the benign cells were seen isolated or in small clumps. Mention has already been made on the nature of cellularity.

2. An important criteria was the size of the cells. All malignant cells were larger than the benign cells. As a rule the larger the cells, higher was the grade of malignancy.

3. The nuclear cytoplasmic ratio was increased in the malignant cells while it was normal in benign cells. Mostly all malignant lesion showed variation in nuclear size and shape with marked pleomorphism in high grade tumour.

4. The nucleoli were more prominent or multiple in the malignant cells.
5. Mitosis figures were infrequently seen, but a deliberate attempt to identify them was not made.

However, none of these criteria are individually pathognomonic of malignancy (Tribe, 1965).Only by assessing all the features of the imprints cells and its correlation with gross and clinical finding a diagnosis of malignancy could be made.

In the present series, imprint cytodiagnosis of 114 cases, showing lymphadenopathy due to inflammatory conditions in 65.8% cases, out of which 15.8% showing chronic lymphadenitis and 50% as tuberculous lymphadenitis, and paraffin section which are taken as a final diagnosis also showing inflammatory lesions in 65.8% as imprint cytodiagnosis but these inflammatory lesions include 46.5% of tuberculous lymphadenitis, 11.4% chronic lymphadenitis and 7.9% reactive hyperplasia. We could not made out the diagnosis of reactive hyperplasia with imprint cytology. Agarwal et al (1977), observed the maximum (68.0%) cases of lymphadenitis are due to inflammatory lesion followed by secondary neoplasm (metastasis) and maximum cases in inflammatory lesions were due to chronic lymphadenitis followed by tuberculous lymphadenitis.

The second important cause of lymphadenopathy was due to primary neoplasm including Hodgkin's lymphoma and Non-Hodgkin's lymphoma (18.4%).

The remaining cause of lymphadenopathy was due to secondary neoplasm, which included metastasis of squamous cell carcinoma, adenocarcinoma, mucoid adenocarcinoma and myeloid leukaemic cell infiltration.

Out of 114 cases of the present series, with histopathological diagnosis, we found 75 had benign lesion, 19 were of primary tumour or lymphoma and 18 were metastasis from malignant neoplasm elsewhere in the body and in miscellaneous group single case of angio-follicular lymphnode hyperplasia and benign sinus histio-cytosis was seen.

By imprint cytodiagnosis of 114 cases of lymph adenopathy revealed 18 of chronic lymphadenitis, 57 of tuberculous lymphadenitis, 6 of Hodgkin's lymphoma, 15 of non-Hodgkin's lymphoma in which 7 cases of lymphocytic lymphoma, 3 cases of histiocytic-lymphocytic lymphoma (mixed cell type); 4 cases of lymphoreticular lymphoma and one case of lymphoblastic lymphoma poorly differen-tiated was seen. In metastatic group 6 cases were of metastatic epidermoid carcinoma, 7 of adenocarcinoma

4 of mucoid adenocarcinoma and one was of myeloid leukaemic cell infiltration. The accuracy rate of these cases were chronic lymphadenitis 55.6%, tuberculous lymphadenitis 87.7%, Hodgkin's lymphoma 100%, metastatic lymphnode 100% and in cases of non-Hodgkin's lymphoma. Accuracy rate was 86.7%. The over all accuracy rate was 85.1%.

The imprints of chronic lymphadenitis were mistaken for tuberculous lymphadenitis, reactive hyperplasia and angiofollicular lymphnode hyperplasia imprint of tuberculous lymphadenitis were mainly mistaken for chronic lymphadenitis, benign sinus histiocytosis where as non-Hodgkin's lymphoma were mistaken for reactive hyperplasia. In cases of Hodgkin's lymphoma, the presence of characteristic Reed Sternbergh cell contributed for 100% diagnosis.

Many worker's studied imprints cytology and compared it with paraffin section technique (Morrison et al, 1952; Lucas, 1955; Aust et al, 1971; Agarwal et al, 1977; Suen et al, 1978, Tyagi et al, 1981; Nagpal et al, 1982; Verma et al, 1983; Chaudhary, 1984, Ademiloyi,1986; Gupta et al, 1988 and Anuradha et al, 1989) and these comparative figures have been presented in (Table-X) and discussed in following paragraphs.

TABLE - X : Showing comparative accuracy rate (%) of different workers in prospect of correlation of cytodiagnosis with histopathological diagnosis.

S.No.	Author's name and year	Chronic lymph-adeni-tis	Tuber-culous lymph-adeni-tis	Reactive hyper-plasia	Non Hodg-kin's lym-phoma	Hodg-kin's lym-phoma	Metas-tatic tumour	Over all accu-racy
1.	Morrison et al, 1952	90.0	-	-	80.0	83.0	100.0	-
2.	Lucas et al,1955	58.3	16.5	-	76.9	29.4	100.0	-
3.	Ultmann et al, 1958	92.7	100.0	-	100.0	90.9	93.9	93.1
4.	Shankaran and Reddy, 1970	69.2	60.8	66.6	100.0	60.0	79.0	79.0
5.	Aust et al, 1971	100.0	80.7	-	100.0	-	100.0	-
6.	Agarwal et al, 1977	100.0	100.0	-	100.0	71.4	94.4	97.6
7.	Suen et al, 1978	83.0		-	88.0		96.0	-
8.	Tyagi et al,1981	-	89.2	-	100.0		100.0	73.6
9.	Nagpal et al,1982	60.0	95.4	-	100.0	100.0	100.0	94.0
10.	Verma et al, 1983	80.0	73.9	83.3	87.5		100.0	83.0
11.	Choudhary, 1984	-	100.0	91.6	80.0	85.7	100.0	90.4
12.	Ademiloyi et al, 1986	50.0	30.77	-	40.0	91.6	84.6	66.0
13.	Gupta et al,1988	81.57	88.9	-	76.9		62.5	84.39
14.	Anuradha et al, 1989	100.0	100.0	-	100.0	87.5	100.0	94.0
15.	Present study, 1990	55.6	87.7	-	86.7	100.0	100.0	85.1

In our study the over all accuracy was 85.1% with imprint cytodiagnosis. Nearly similar accuracy rate have been reported viz 93.1% (Ultmann et al, 1958), 79% (Sankaran and Reddy, 1970), 97.6% (Agarwal et al, 1977), 94% (Nagpal et al, 1982), 83% (Verma et al, 1983), 66% (Ademiloyi, 1986), 84.39% (Gupta et al, 1988), and 94% (Anuradha et al, 1989).

The cytological criteria for diagnosis of chronic lymphadenitis was increased in cellularity of the imprint smear together with presence of few neutrophils, plasma cells, and eosinophils. Similar observations have also been made by Lucas (1955), Ultmann et al (1958), Agarwal et al (1977), Nagpal et al (1982), Anuradha et al (1989). The accuracy rate in case of chronic lymphadenitis in the present series was 55.6%, however several other workers reported the accuracy rate varying from 50 to 100% (Morrison et al, 1952; Lucas, 1955; Ultmann, 1958; Shakaran and Reddy, 1970; Aust et al, 1971; Agarwal et al, 1977; Nagpal et al, 1982; and Verma et al, 1983; Chaudhary, 1984; Ademiloyi, 1986; Gupta et al, 1988; and Anuradha et al, 1989).

In our study we found that 57 cases diagnosis as tuberculous lymphadenitis but histopathologically only 53 (46.5%) cases of tuberculous lymphadenitis were confirmed, Agarwal et al 1977 reported 32% of the patient has tuberculous lymphadenitis which were histologically proved, but,

Pamra and Mathur reported the incidence of tuberculosis as 70.6% in a large series of 303 cases of cervical lymphadenopathy. Since the respiratory route of tuberculosis infection predisposing the cervical lymphnodes to scading with tubercle bacilli, the increased incidence of tuberculosis in cervical lymphnode is easily explained in this study also. Cervical tuberculous lymphadenitis was higher in 24 cases than generalized tuberculous lymphadenitis in 8 cases.

The tuberculosis patient had a age of 35 years with male female ratio of 1:4. Gupta et al reported lymph node tuberculosis at a mean age of 33 years with a male to female ratio of 2:1, while compbell and Dyson reported lymphnode tubercular at a mean age of 35 years but in the female prepondarance from our study we concluded that tubercular lymphadenitis was more commonly seen in female than male in this region and a large majority of cases were between 11 to 30 years age group. Similar result was reported by Pamra and Mathur (1974).

Lucas (1955), Ultmann et al (1958), Aust et al (1971), have stated that diagnosis of tuberculosis could be made on the demonstration of epitheloid cells with occasional ddemonstration of acid fast bacilli. From the present study, it was felt that where there was hypocellularity, associated with the granular material in the imprint smear, the diagnosis of tuberculous lymphadenitis should be

suspected and a meticulous search would always result in spotting out group of epitheloid cells and giant cells. Acid fast bacilli could not be demonstrated by special stain in an any of the imprints smear of tuberculous lymphadenitis in our study.

Agarwal et al (1977) have demonstrated acid fast bacilli only in 3 out of 40 instances.

In the present study, the accuracy rate in case of tuberculous lymphadenitis was 87.7%, almost similar accuracy has been observed by Aust et al (1971), Verma et al (1983), and Gupta et al (1988). However the accuracy rate as low as 16.5% found by Lucas (1955), and as high as 100% (Ultmann et al, 1958; Agarwal et al, 1977; Choudhary, 1977; Anuradha et al, 1989) is also have been reported.

Reactive hyperplasia in our study by paraffin section diagnosed in 9 (7.9%) cases. But with imprint cytology I failed to diagnosis any single case of reactive hyperplasia. Verma et al (1983) diagnose reactive hyperplasia in 10/12 imprint smears and Choudhary (1984), also found accuracy rate of 91.6% in cases of follicular reactive hyperplasia. The criteria for this diagnosis increased cellularity of the smears with predominance of lymphocytes at various stage of maturation, but in my opinion the reactive hyperplasia diagnosis made by imprint cytology is very difficult. Verma et al (1983) found one false positive case

of reactive hyperplasia which turned out to be Hodgkin's lymphoma and this difficulty was also expressed by Gupta et al (1977). Shankaran and Reddy, (1970) and Verma et al (1983) found accuracy rate in cases of reactive hyperplasia by imprint cytology was 66.6% and 83.3% respectively.

The commonest cause of generalised lymphadenopathy was lymphoma. In Hodgkin's lymphoma the accuracy rate was 100% in our study and this is due to presence of characteristics, Reed Sternberg giant cells in the imprint smears, but all the cases were of mixed cellularity type. The accuracy rate in Hodgkin's lymphoma have been observed by other worker's as 83% Morrison et al (1952), 29.4% by Lucas (1955), 90.9% Ultmann et al (1958), 60% Shankaran and Reddy (1970), 71.4% Agarwal et al (1977), 100% Nagpal et al (1982), 85.7% Choudhary (1984), 91.67% Ademiloyi et al (1986), and 87.5% Anuradha et al (1989).

The low accuracy rate was mainly due to the Hodgkin's disease of nodular sclerosis type and also due to lymphocytic predominance and lymphocytic depletion type. However, the accuracy rate was found to be high by above workers also in mixed cellularity type of Hodgkin's disease. Fortunately our all cases of Hodgkin's lymphoma were of mixed cellularity type in which characteristic Reed Sternberg giant cells and pleomorphism together with the presence of premature of mature reticulam cells seen. In observing

the absence of these specific cells the smears could be mistaken for that of chronic lymphadenitis. The same remark have been expressed by Morrison et al (1952) and Koss (1968) in case of Hodgkin's disease specially of sclorising type. Ultmann et al (1958) gave 90.9%, Suen et al (1978) gave 87.5%, Verma et al (1983) gave 76.92% Gupta et al (1988), accuracy rate were found in lymphoma (Hodgkin's and Non-Hodgkin's type).

In the diagnosis of non-Hodgkin's lymphoma lymphocytic or lymphoreticular type has been quite easy to diagnose by imprints due to marked hypercellularity of the smears together with the monomorphic character of the corresponding cells. The same features have also been stressed by most of the workers (Lucas, 1955 and Ultmann et al, 1958, and Nagpal et al 1982, Agarwal et al 1977). However two cases could not be confirmed by paraffin section technique in present study. False positivity has also reported by Webbs (1978).

Metastatic lesions were also found to be the maximum in axillary lymphonodes followed by cervical lymphnodes. Out of 18 cases, 9 had involvement of axillary followed by cervical (6), and one each for mesentric and inguinal lymphnodes. Leukaemic cell infiltration was associated with generalised lymphadenopathy. Gupta et al (1988) found maximum involvement in cervical followed by axillary

lymphnode. Maximum cases of secondaries were found in older age group (51 to 60 years). None of the case was showing metastasis below 30 years or above 70 years age.

The diagnosis of secondaries in the lymphnodes from a tumour else where in the body present hardly any diagnostic problem. In our imprint cytological smears the accuracy rate in this group was 100%, similar observations were found by various worker like Morrison et al (1952), Lucas (1955), Aust et al (1971), Tyagi et al (1981),Nagpal et al (1982), Verma et al (1983), Ghandhur (1984),Anuradha et al (1989). Primary lesion could be diagnosed easily provided the cells in the smears were well differentiate e.g. in instances of squamous cell carcinoma, adenocarcinoma or mucoid adenocarcinoma. Moore and Reagan (1953) in there study of lymphnode imprint found 20 lymphnodes with metastatic diseases showing 100% correlation between imprint and tissue section.

The problem of diagnosis however persisted in case of leukaemic cell infiltration cause the smears showed pleomorphism apparently similar to that observed in chronic lymphadenitis, but on closer scrutiny a correct diagnosis was possible due to presence of myeloid series of cells in different stages of maturation. The over all accuracy was found with imprint cytodiagnosis in cases of lymphadenopathy as 85.1%.

Near about similar over all accuracy rate by imprint cytodiagnosis were found by Gupta et al 1988 (84.39%), Verma et al 1983 (83.0%), Shankran and Reddy 1970 (79.0%), Tyagi et al 1981 (73.6%) and minimum accuracy rate was recorded by Ademiloyi 1986 (66.0%) and maximum overall accuracy was observed by Chaudhary 1984 (90.4%), Ultmann et al 1958 (93.1%), Nagpal et al 1982 (90.4%), Anuradha et al 1989 (94.0%) and Agarwal et al 1977 (97.6%).

In conclusion we can say that imprints smear method is reliable and dependable procedure for diagnosing various lymphnode diseases.

Both imprints cytology and frozen section technique were found to be reasonably simple and quick Godwin (1976) advocated the use of haematoxyline and eosin for in conclusive cytological diagnosis with other stain. Frozen section took a little more time before a diagnosis was ventured because we used a cryostat, a new modification of the conventional freezing microtome, taking about 15 minutes and using haematoxyline and eosin stains. The use of the same stain helped in comparing the two methods. The slides prepared by cryostat were however of superior quality and thin (5-6 microns), thereby making possible high power microscopic details to he observed with case. Another advantage of this method was that multiple process of fresh

frozen tissue from different sites were processed simultaneously thus reducing the chances of sampling errors. These finding are similar to concurred by Horn (1962) and Sparkman (1962).

In present study, frozen section done of all the 114 cases of lymphnode with help of cryostat and found 77 cases (67.5%) of lymphadenopathy were due to inflammatory lesions in which chronic lymphadenitis were seen in 14 (12.2%), tuberculous lymphadenitis in 53 cases (46.5%) and reactive hyperplasia in 10 cases (8.8%). In primary neoplasm 19 (16.7%) and secondaries neoplasia 18 (15.8%), the results are same as compare the paraffin section.

In cases of chronic lymphadenitis out of 14 cases only 13 cases were confirmed by paraffin section and one case was misdiagnosed which was confirmed by paraffin section as a angiofollicular lymphnode hyperplasia, thus accuracy rate was found in case of chronic lymphadenitis was 92.8%. All the 53 case of tubercular lymphadenitis were diagnosed by frozen section method and were confirmed by paraffin section. Thus in tuberculous lymphadenitis the accuracy rate was 100%. 10 cases diagnosed as a reactive hyperplasia with frozen section technique in which only 9 cases were confirmed with paraffin section, one case misdiagnosed by frozen section was

confirmed as benign sinus histiocytosis with paraffin section. Thus accuracy rate in cases of reactive hyperplasia with frozen section technique was found to be 90%. All the cases of Hodgkin's lymphoma and non-Hodgkin's lymphoma and metastatic lymphnode were diagnosed correctly, thus the accuracy rate was 100% in lymphoma and metastasis including myeloid leukaemic cell infiltration. The overall accuracy rate was found to be 98.2% by frozen section technique. Other workers have reported accuracy rate as 97.1% (Kaufman et al, 1986), 93% (Chaudhary, 1984) and 98.0% (Ackerman and Ramirej, 1959). Godwin (1976) used the scrapping technique for 20 years and lathough the reports no figures, his statement indicate that he prefer imprints to frozen section.

Bloustein and Silberberg 1977 studied 21 benign lymphnodes, 23 with metastatic carcinoma and 4 with lymphoreticular neoplasm. They state that a small focus of cancer in a lymphnode can be missed by frozen section with the conventional method of sampling the nodes.

Sakai and Lauslathi (1969) in comparing and analysing the results of cytodiagnosis and frozen section during operation attained an accuracy of 95.7% by frozen section and 95.5% by imprint cytology. Pickren and Burke (1963) in a similar type of study attained combined accuracy of 97.4% but did not give statistical analysis of

their 1819 cases. Suen et al (1978) accomplished a combined accuracy of 98.3% and an overall accuracy rate of 93.6% by imprints. Thus the accuracy rate in our series compared favourably with the rate of other authors.

Chaudhary (1984) also studied 52 unselected lymph node biopsies with imprint cytology and frozen section and the results were compared with paraffin section, and found accuracy of frozen section diagnosis of lymphnode was 93% more than imprint cytology accuracy rate (90.4%).

Frozen section in conjunction with imprint cytology should be used where gross examination findings are equivocal, like in well differentiated carcinoma, or where peroperative diagnosis has an immediate bearing on the surgical modification; where immediate interference is not contemplated, only imprints would suffice. Sakai and Lauslathi (1969), Suen et al (1978), Pickerman Burke (1963) and Bamforth and Osborn (1958) all advocate adjuvant cytology to frozen section because is helps in establishing nature of pathological entity, avoids false decision, ensures accuracy in rapid tissue diagnosis and reduce sampling error. Tribe (1965) concluded that tumour imprints would probably never replace first class frozen section, especially those produced on cryostat, but suggested that they have a definite place in combination with frozen section.

We concur with the findings of various other authors that the high degree of accuracy by frozen section with cryostat in our series and a overall accuracy of 98.2% suggest that the smear or imprint cytological diagnostic method are not as reliable as cryostat frozen section. It is believed that by a more meticulous technique, and with experience, the accuracy of imprints cytology can be improved further.

Considering its somplicity, universal application, economical advantages and the absence of false positive in our results, we strongly recommended that it should be used universaly in all hospitals as a routine parts of surgical interference; practioned in an operating suite, even surgical residant and house surgeons tought to interpret the common malignant tumour in the light of gross study.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The study for evaluation of imprint cytodiagnosis in cases of lymphadenopathy and comparative study with frozen section and paraffin section was conducted on the patients having lymphadenopathy attending the out patient departments as well as admitted cases in the wards of M.L.B. Medical College Hospital, Jhansi, over period of one year.

Lymphnode is highly cellular organ, so that any delay in the fixation results in poor preservation and thick sections. Various methods for quick diagnosis of lymphadenopathy have developed that include fine needle aspiration cytology, imprint cytology and frozen section technique. The imprint cytodiagnosis was described initially by Berman (1953) and histopathological finding were correlated with that of imprints smears, where as frozen section technique was first popularised by Wilson (1905).

In the present study, both the technique for rapid diagnosis (imprint cytodiagnosis and frozen section technique) have been used and compared with paraffin section technique. 114 cases of either sex and all age groups were studied which included cervical, axillary, mesentric, inguinal lymphnodes and generalised lymphadenopathy. Most of the cases were of cervical lymphadenopathy. Diagnosis by paraffin section technique was taken as final. As per final diagnosis maximum number of cases were contributed by inflammatory

lesions followed by primary lymphoid neoplasm and secondary neoplasm. In inflammatory lesions group highest incidence was of tuberculous lymphadenitis followed by chronic lymphadenitis and reactive hyperplasia. In primary neoplasm group most of the cases were of non-Hodgkin's lymphoma and few Hodgkin's lymphoma. In metastatic carcinoma group most of the cases were of secondary adenocarcinoma followed by squamous cell carcinoma and mucoid adenocarcinoma; one case of myeloid leukaemic cells infiltration was also encountered. There was one case of angiofollicular lymphnode hyperplasia and one of benign sinus histiocytosis.

By imprint cytodiagnosis, out of 114 cases 75 cases of inflammatory lesion, 21 cases of primary neoplasm and 18 cases of secondary neoplasm were diagnosed. By imprint cytodiagnosis, the accuracy rates were 100% for Hodgkin's lymphoma and metastatic carcinoma, while 87.7% for tuberculous lymphadenitis, 86.7% for non Hodgkin's lymphoma and 55.6% for chronic lymphadenitis. Some cases of chronic lymphadenitis were misdiagnosed and proved to be reactive hyperplasia, tuberculous lymphadenitis and angiofollicular lymphnode hyperplasia, while some cases of tuberculous lymphadenitis were confirmed as chronic lymphadenitis, reactive hyperplasia and benign sinus histiocytosis. Two cases of non-Hodgkin's lymphoma were confirmed as reactive hyperplasia by paraffin section technique. The over all accuracy rate by imprint cytodiagnosis came out to be 85.1%.

By frozen section technique 77 cases of inflammatory lesion, 19 cases of primary neoplasm and 18 cases of secondary neoplasm were observed. Amongest 77 cases of inflammatory lesion maximum number of cases of tuberculous lymphadenitis followed by chronic lymphadenitis and reactive hyperplasia; while in primary neoplasm group maximum cases of non-Hodgkin's lymphoma followed by Hodgkin's lymphoma were observed. The distribution of secondary neoplasm groups was same as that of imprint cytodiagnosis and paraffin section technique.

By frozen section technique, the accuracy rate was 100% for primary and secondary neoplasm and tuberculous lymphadenitis 92.8% for chronic lymphadenitis and 90% for reactive hyperplasia. The overall accuracy rate for frozen section technique came out to be 98.2%. Only 2 cases were misdiagnosed by this technique, which came out to be angiofollicular lymphnode hyperplasia and benign sinus histiocytosis when compared with paraffin sections.

In present study the overall accuracy rate by imprint cytodiagnosis was 85.1%, where as various workers reported accuracy rates ranging from 66% to 97.6%(Ultamann et al 1958, Shankran and Reddy 1970, Agarwal et al 1977, Tyagi et al 1981, Nagpal et al 1982, Verma et al 1983, Choudhary 1984, Ademiloyi et al 1986 and Anuradha et al 1989).

The accuracy rate, by many workers and also in the present study was remarkably higher for metastatic carcinoma, Hodgkin's lymphoma and Non-Hodgkin's lymphoma. The accuracy rate came out to be lower in present study as well as by other worker's for chronic lymphadenitis. The accuracy rate was reasonably good for Non-Hodgkin's lymphoma and tuberculous lymphadenitis. Few workers also classified reactive hyperplasia by imprint cytodiagnosis, but in present study we could not made differentiation between chronic lymphadenitis and reactive hyperplasia.

The overall accuracy rate by frozen section technique was 98.2% which is comparable with studies of other workers (Ackerman and Ramirej 1959, Choudhary 1984 and Kaufman et al, 1986).

So in our study, we conclude that imprint cytodiagnosis is rapid and reasonably reliable technique for lymphadenopathies. This technique is practically equal as paraffin technique for primary and secondary neoplasm. However for inflammatory lesion, we can not depend on imprint cytodiagnosis. Considering its simplicity this technique should be followed and should be accepted particularly to exclude malignancy. The frozen section technique is equally good as paraffin section technique and should be employed routinely where the facilities of cryostat are available. During our study we have discovered following

facts which can be advocated for improving the diagnosis of lymphadenopathy by imprint cytology and frozen section.

The study has revealed that :-

1. Universal applicability of all type of lesion from the lymphnode by both imprint and frozen section.
2. Per operative diagnosis should be practised in an operating suite thereby facilitating constructive communication between surgeon and pathologist.
3. Frozen section diagnosis on cryostat is a highly accurate method of diagnosis permitting high power microscopic cetails of their section to be studied with ease.
4. Imprint cytology serves as a reliable, cheap and time saving alternative to frozen section.
5. Imprint cytological study can be carried out in an averagely equipped community hospital.
6. Where facilities permit and particularly in equivocal are ususual lesions, where immediate surgery is contemplated, adjuvant cytology to frozen section should be employed. Both method compliment each other and should not be consider as separate entities.
7. Imprint cytodiagnosis should be included as a part of surgical residency training.

8. Multiple imprint from different areas and correct technique, both learnt by experience, can improve accuracy by reducing sampling errors.

9. Representative tissue from the specimen sent for histopathology should be taken for both imprint and frozen section preparations.

10. A larger number of benign cases must be studied from lymphadenopathy, particularly inflammatory lesion to further validate and substantiate the findings.

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A P P E N D I X

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WORKING PROFORMA

"EVALUATION OF IMPRINT CYTODIAGNOSIS IN CASES OF LYMPH-ADENOPATHY AND COMPARATIVE STUDY WITH FROZEN SECTION AND PARAFFIN SECTION"

1. Serial No._____ MRD/OPD No._____

2. Patient's Name_____ Age/Sex _____

3. Address _____ Ward/Bed No._____

_____ Date of Admission____

4. Surgeon Incharge Dr._____

5. Clinical diagnosis _____

6. Relavant clinical data

(A) Chief complaint :

(B) General Examination Systemic Examination

(C) Local site

(D) Distant lymphnode

(E) Liver

(F) Spleen

7. Relavant Haematological investigations (If done) :

T.L.C. Haemoglobin _____

D.L.C. E.S.R. _____

G.B.P.

8. Previous histopathological examination (If any)

9. Tissue removed on _____

10. Pathological Examination

(A) Gross Examination :

- Size and shape
- Capsule
- Consistency
  - Soft
  - Rubbery
  - Firm
- Cut surface
- Necrosis
- Haemorrhage

(B) Imprint cytodiagnosis :

- Serial No.
- Fixative used - Equal volume ether and alcohol
- Staining used - 1- Haematoxyline and Eosin.
  2- Papanicolaou's
  3- Zeil Neelson staining (For acid fast bacilli)

(C) Diagnosis by frozen section with cryostat microtome-
- Serial
- Staining use :
  - Haematoxyline and Eosin

(D) Diagnosis by paraffin section :

- Histopath No._____
- Fixative used - Buffered formal saline
- Staining used - Haematoxyline and Eosin and special staining like Reticulin, PAS where ever required.

11. Final diagnosis :
